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Research Article

POTENTIAL MODULATION OF DISEASE ACTIVITY INDICES AND INFLAMMATORY BIOMARKERS IN PATIENTS WITH ACTIVE INFLAMMATORY BOWEL DISEASE ON MELATONIN ADJUVANT THERAPY

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ABSTRACT

Objective: The objective of this study was to explore the benefits of melatonin supplementation in the active stage of moderate to severe inflammatory bowel diseases (IBDs) as an adjuvant to the standard immunosuppressant and biological targeting therapy in both Crohn's disease (CD) and ulcerative colitis (UC) patients.

Methods: This is an interventional prospective randomized controlled, open-label, single-center study was carried out on 50 patients visiting the inpatient biology unite diagnosed as moderate-severe active IBD. Melatonin adjuvant was added to the standard treatment.

Results: Disease activity indices showed significant improvement in both diseases after melatonin adjuvant therapy compared to the standard treatment alone (CD −61.80 vs. 36.48%), (UC −74.48% vs. −46.66%) ($p<0.01$). The erythrocyte sedimentation rate (ESR) level showed higher percent of reduction after melatonin adjuvant in both diseases ($p<0.05$). CD patients presented with slight reduction in matrix metalloproteinases-9 (MMP-9) level following on melatonin adjuvant therapy compared to standard therapy ($p>0.05$). At baseline, a significant correlation between severity scores of both diseases and both ESR and MMP-9 in UC patients, but after treatment, only the ESR level was correlated to the improvement in disease score in CD patients.

Conclusion: Melatonin produced marked improvement in the disease activity status in both CD and UC after receiving melatonin adjuvant in active stage of disease, and notable modulation of inflammatory biomarkers with no clear role on MMP-9 expression.

Keywords: Inflammatory bowel disease, Oral melatonin, Disease activity indices, Matrix metalloproteinases, Erythrocyte sedimentation rate.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are idiopathic, chronic, and relapsing inflammatory disorders of the gastrointestinal tract (GIT) affecting many people in either developed or developing countries. Its incidence is increasing progressively [1]. Patients with both Crohn's disease (CD) and ulcerative colitis (UC) need a long-term combination therapy to control but not completely cure the disease. Most of the medications used for the management of IBD were disappointing because they are moderately ineffective, monotargeted, have numerous adverse effects, and too expensive for many people [2,3]. Thus, compounds that have multiple molecular target pathways and more affordable have promising potential for the treatment of IBD such as melatonin, a well-known endogenous hormone, which has a potential beneficial effects in IBD because of its ability to modify some molecular mechanisms of oxidative stress, inflammation, cellular injury, and fibrosis [1,3]. Its presence in the GIT was approved in 1977, and the amount of melatonin is measured to be 400 times higher than that in the pineal gland [4].

Melatonin exerts its physiological action through specific membrane receptors, which are melatonin-1 receptor (MT1), MT2, and MT3 [5]. These receptors can be located in the GIT and their participation in the control of GI inflammation, motility, and pain has been stated in several basic and clinical studies [3,6]. Melatonin acts as an immunological buffer to describe the pleiotropic, diverse, and complex actions of melatonin on the immune system in the most accurate way [7]. Melatonin might act as an immunostimulant under acute or immunosuppressed situations, providing a pre-activated status for a more efficient primary immune response to exterior stressors such as parasites and viruses [8]. Nevertheless, in the existence of a transient or chronic aggravated immune response, such as septic shock, melatonin might impact a negative regulation and could be assumed to act as an anti-inflammatory molecule [7]. The most predominantly observed effects of melatonin were on reduction of CAM, nuclear factor κB (NF-κB) stimulation, inducible nitric oxide synthase suppressing, and suppression of macrophages. All are causing positive modulation of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, malondialdehyde, nitric oxide, myeloperoxidase, leukocyte infiltration, and cyclooxygenase-2 pathway, and melatonin was capable of raising glutathione and superoxide dismutase levels [9]. Furthermore, melatonin reduces bacterial translocation, fibrosis, apoptosis, and decrease matrix metalloproteinases (MMPs) activities in GIT. Since most of the inflammatory pathways were connected to each other, therefore, melatonin probably has a major complex effect through an improvement in oxidative/inflammation pathway [1,3].

Melatonin has gastroprotective effects which are observed using it in many GIT problems, and one of these problems is gastroesophageal reflux disease (GERD) [10]. Moreover, melatonin was used in the treatment of gastric and duodenal ulcers by enhancing the healing of *Helicobacter pylori*-infected gastroduodenal ulcer; an effect probably mimics other supplements such as curcumin or aloe vera through possessing antioxidant and anti-inflammatory effects [11-13]. Melatonin was proposed to be a hopeful future medication with a potential for the management of irritable bowel syndrome. This is because it was efficacious in relieving pain and increasing pain threshold in those patients [14].

Melatonin has the ability to protect GIT mucosa against injury by enhancing the immune system, enhance microcirculation, and epithelial regeneration [15].

Addressing this new advent, clinical studies could be a proper approach for a clear definition of the entitled effect of melatonin in GIT disease. Klupinsk *et al.,* in 2006, noticed a relatively lower melatonin concentration in blood of patients with an erosive form of GERD, ulcer-like functional dyspepsia, and duodenal ulcer disease [16].

Taking into account all the previous evidence, melatonin potentially plays a key role in modulating the pathogenesis and development of IBD. However, no previous study was conducted to explore the benefits of melatonin supplementation in the active stage of moderate to severe IBD as an adjuvant to the standard immunosuppressant and biological targeting therapy in both CD and UC patients. Accordingly, this study was designed to assess the potential role of melatonin adjuvant in ameliorating disease process both subjectively and objectively in patients with moderate-severe active IBD in combination with the standard therapy.

METHODS

Study design

This is an interventional prospective randomized controlled open-label study was conducted at Baghdad teaching hospital and was carried out during the period from August 2016 to March 2017. The protocol was achieved under the supervision of specialist physicians, and patients were treated according to clinical practice guidelines and disease severity [17,18]. Informed written consent was obtained from all participants, and this study was approved by the Ethics Committee of Mustansiriyah University, College of Pharmacy.

Patients

A total of 50 ambulatory adult candidate patients aged over 18 years presented with active moderate to severe IBD were enrolled in the study during their visit the inpatient biology unite. The CD patients were diagnosed with moderate-severe active stages according to international definitions of diseases "activity" in CD by CD Activity Index (CDAI) [19]; meanwhile, UC patients were diagnosed with moderate to severe active UC according to Truelove and Witts' severity index [20].

Patients were excluded if they had the following: Night shift workers, diagnosed alcohol or narcotic dependence, currently using anticoagulant drugs or any history of bleeding disorder, current intake of melatonin, iron, antioxidants and hypnotics, on current renal dialysis, patients with active liver disease, patients with immune system diseases, patients with hypoparathyroidism or hyperparathyroidism, patients on systemic or rectal corticosteroids within the last 8 weeks, pregnant and lactating women, poorly controlled medical conditions (e.g., uncontrolled diabetes) or under current therapy, and known hypersensitivity to melatonin.

Patients were randomly assigned into four main groups according to the study intervention; Group 1 includes 15 patients with active moderate to severe CD assigned to receive the standard treatment; Group 2 includes 15 patients with active moderate to severe CD assigned to receive standard treatment with 5 mg melatonin adjuvant tablets; Group 3 includes 10 patients who have moderate to severe UC assigned to receive standard treatment; and Group 4 includes 10 patients who have moderate to severe UC assigned to receive standard treatment with 5 mg melatonin adjuvant tablets. All patients received the treatment for 8 weeks' regimen.

The standard treatment given to patient with CD includes infliximab (Remicade® 5 mg/kg) iv infusion every 8 weeks and azathioprine (Imuran® 50 mg) tablet twice daily. The standard treatment given to patient with UC includes infliximab (Remicade® 5 mg/kg) iv infusion every 8 weeks, azathioprine (Imuran® 50 mg) tablet twice daily, and mesalamine (Pentasa®) 1 g 4 times daily. A daily dose of 5 mg melatonin adjuvant tablets at bedtime was added to CD patients and to patient with UC who were already received three or more doses of infliximab (infliximab iv induction dose of 5 mg/kg at 0 weeks, 2 weeks, and 6 weeks, followed by a maintenance dose of 5 mg/kg every 8 weeks).

Materials and Methods

Safety monitoring

At each visit, from week 0 to week 8, patients underwent a physical examination, vital signs assessment, and recording previous or concomitant medications. AEs were recorded and general laboratory tests, including urinalysis, were performed.

Disease activity scoring

Estimating the level of Harvey-Bradshaw index (HBI) [21] for patients with CD and the simple clinical colitis activity index (SCCAI) for patients with UC [21] was performed at week 0 and after 8 weeks at the end of the study. The total index score of HBI is as follows: Remission (<5), mild activity (5–7), moderate activity (8–16), and severe activity (>16). On the other hand, the total index score of SCCAI ranges from 0–19.

Laboratory analyses

About 3 ml was collected in k3 EDTA tube and used in estimating the erythrocyte sedimentation rate (ESR) using automated assay using the Mixrate-X20 instrument. The results are complete within 30 min, correlated to 1 h following the Westergren reference method. The reference range is ≤15 mm/h for men and ≤20 mm/h for women [22]. The MMP-9 concentration is determined using ELISA test. The principle of this test was based on the sandwich type. The absorbance was estimated at a wavelength of 450 nm. Then, MMP-9 concentration in the samples was detected proportionally and is determined using the standard curve [23].

Statistical analysis

The SPSS 24.0 was used to make the statistical analysis. $p>0.05$ is not statistically significant while $p<0.05$ statistically significant and $p<0.01$ are highly statistically significant. Paired t-test is statistically used to compare between pre- and post-treatment results in same group. Two-sample t-test is used to compare pre- or post-treatment between Group 1 and Group 2 patients. Two-way ANOVA repeated measure is used to get p value of interaction between the groups.

RESULTS

Baseline characteristics

The present study included 30 CD patients (10 females [33.3%] and 20 males [66.7%]). The age range for all patients was between 18 and 53 years with the average age of the study groups was 33.60±10.28 years for Group 1 patients and 35.13±7.92 years for Group 2 patients. The average body mass index (BMI) for Group 1 patients and Group 2 patients were 23.43±5.39 kg/m^2 and 24.72±3.38 kg/m^2, respectively. The mean duration of the disease was 3±2.48 years and 3.07±2.15 years for Group 1 patients and Group 2 patients, respectively. No significant difference was found between the study groups concerning all CD patients demographic data ($p>0.05$) (Table 1).

The present study also included 20 UC patients (5 females [25%] and 15 males [75%]). The age range for the groups was between 18 and 57 years with the mean age of the study groups was as follows: Group 3 patients 35.10±13.03 years and Group 4 patients 36.70±8.12 years. The mean BMI for Group 3 patients was 23.24±1.99 kg/m^2 and was 23.54±4.09 kg/m^2 for Group 4 UC patients. The overall mean duration of the disease for Group 3 and Group 4 patients, respectively, was 4.4±2.17 and 4.0±2.54 years. No significant difference was found between the study groups concerning all UC patients demographic data ($p>0.05$) (Table 2).

Effect of standard treatment and melatonin adjuvant therapy on disease activity indices

Subjective data analysis revealed no significant differences in the mean HBI score between Group 1 and 2 CD patients at baseline ($p>0.05$); however, statistically significant differences were found after 8 weeks of treatment between both groups ($p<0.05$). Moreover, post-treatment in both study groups, highly significant decrease in mean HBI score was noticed compared to pre-treatment level ($p<0.01$), significant down

ceiling percentage of change was seen in Group 2 patients on melatonin supplement (−61.80%) compared to Group 1 patients (−36.48%), and overall, there were significant differences between the study groups at the end of study period (p value of interaction=0.021) (Table 3). Patients with UC showed significant difference in the mean SCAI score between the two groups after 8 weeks of treatment (p<0.05), and in both groups, there was a highly significant decrease in the mean SCAI score after 8 weeks of treatment when compared to pre-treatment level (p<0.01), with significant up ceiling percentage of change in Group 4 patients compared to Group 3 patients (−74.48% vs. −46.66%), respectively. Accordingly, the overall result showed a significant difference between the patient groups at the end of study period (p value of interaction=0.024).

Effect of standard treatment and melatonin adjuvant therapy on inflammatory biomarkers

Data analysis of the present study demonstrate that there was no significant difference in the mean ESR between Group 1 and 2 CD patients at baseline and also after treatment, nevertheless, significant decrease in mean ESR level was found those patients were seen after 8 weeks of treatment compared to pre-treatment level in each group (p<0.05), and the up ceiling percentage of change was noticed in patients on melatonin adjuvant therapy (−49.2%) compared to group 1 patients (−28.1%). Still, there was no significant difference between the groups at the end of study period (p value of interaction=0.242) (Table 4).

Table 1: Patients demographic and disease characteristics of Crohn's disease patients

Study groups			
Variable	**Group 1**	**Group 2**	**p value**
Gender	n (%)	n (%)	
Female	7 (46.7)	5 (33.3)	0.456^{NS}
Male	8 (53.3)	10 (66.7)	
Total	15 (100)	15 (100)	
Age (year)	33.60± 10.28	35.13 ± 7.92	0.651^{NS}
BMI (kg/m^2)	23.43± 5.39	24.72± 3.38	0.436^{NS}
Duration of disease (year)	n (%)	n (%)	
≤2	8 (53.3)	7 (46.7)	
2–4	3 (20)	6 (40)	0.936^{NS}
4–6	2 (13.3)	0 (0)	
≥7	2 (13.3)	2 (13.3)	

Data presented as mean±SD. Number of patients (n), percentage (%), NS: No significant differences. Two-sample *t*-test is used for statistical analysis of (age, BMI). Chi-square test is used for statistical analysis of (gender, duration of the disease). BMI: Body mass index, SD: Standard deviation

Table 2: Demographic and disease characteristics of ulcerative colitis patients

Study groups			
Variable	**Group 3 n (%)**	**Group 4 n (%)**	**p value**
Gender	n (%)	n (%)	-
Female	3 (30)	2 (20)	0.606^{NS}
Male	7 (70)	8 (80)	
Total	10 (100)	10 (100)	
Age (year)	35.10±13.03	36.70±8.12	0.746^{NS}
BMI (kg/m^2)	23.24±1.99	23.54±4.09	0.834^{NS}
Duration of disease (year)			
≤2	3 (30)	3 (30)	0.709^{NS}
2–4	2 (20)	3 (30)	
4–6	3 (30)	2 (20)	
≥7	2 (20)	2 (20)	

Data presented as mean±SD. Number of patients (n), Percentage (%), NS: No significant differences. Two-sample *t*-test is used for statistical analysis of (age, BMI). Chi-square test is used for statistical analysis of (gender, duration of the disease). BMI: Body mass index, SD: Standard deviation

The results of the present study revealed no marked change in the mean MMP-9 level at baseline and after treatment between Group 1 and 2 CD patients (p>0.05). After treatment, theme an MMP-9 did not show any decrease in its level compared to pre-treatment in both study groups (p>0.05), and the overall result revealed that there was no significant difference among CD patients in respect to MMP-9 level at the end of study (p value of interaction=0.952).

In UC patients, no significant difference in the mean ESR between Group 3 and Group 4 patients' pre-treatment and post-treatment (p>0.05), nevertheless, a significant decrease was found in Group 4 UC patients on melatonin adjuvant only after 8 weeks compared to pre-treatment level (p<0.05). Still, no significant difference was found at the end of the study (p value of interaction=0.151) (Table 5).

Similar to the CD patients, the mean MMP-9 level did not show significant difference neither between Group 3 and 4 UC patients at baseline and after treatment (p>0.05) nor after treatment compared to pre-treatment in study Groups 3 and 4 (p>0.05). The net result showed no significant difference between the patient groups at the end of study (p value of interaction=0.290).

The correlation between the disease activity indices and inflammatory biomarkers in both diseases

At baseline, the Pearson correlation coefficient produced a significant correlation between severity scores of both diseases and ESR (p<0.05), yet after both treatments, only the ESR level was significantly correlated to the improvement in disease activity score in CD patients. The MMP-9

Table 3: Effect of standard treatment and melatonin adjuvant therapy on disease activity indices in CD and ulcerative colitis patients

Variable	Study groups in CD		
	Group 1	**Group 2**	**p value**
HBI			
Pre-treatment	12.06±5.98	12.20±4.61	0.946^{NS}
Post-treatment	7.66±4.49	4.66±2.05	0.029*
p value	<0.001**	<0.001**	0.021^{a}*
Percent change	−36.48%	−61.80%	-
SCAI			
Pre-treatment	10.50±4.06	9.80±4.10	0.706^{NS}
Post-treatment	5.60±3.43	2.50±2.06	0.025*
p value	<0.001**	<0.001**	0.024^{a}*
Percent change	−46.66%	−74.48%	-

Data presented as mean±SD. ^{a}Is p value of interaction. NS: No significant differences (p>0.05), (*) Significant difference (p<0.05), (**) Highly significant difference (p<0.01). CD: Crohn's disease, HBI: Harvey-Bradshaw index

Table 4: Effect of standard treatment and melatonin adjuvant therapy on ESR and MMP-9 in CD patients

Variable	Study groups in CD		
	Group 1	**Group 2**	**P value**
ESR (mm/h)			
Pre-treatment	25.13± 20.59	24.80± 16.55	0.961^{NS}
Post-treatment	18.06 ± 13.72	12.60± 7.16	0.186^{NS}
p value	0.027*	0.002**	0.242^{NS}
Percentage change	-28.1%	-49.2%	-
MMP-9			
Pre-treatment	1964.86±196.05	1914.46±225.55	0.519^{NS}
Post-treatment	1939.80±192.47	1893.73±226.42	0.553^{NS}
p value	0.732^{NS}	0.660^{NS}	0.960^{aNS}
Percent change	−1.27%	−1.08%	-

Data presented as mean±SD. ^{a}Is p value of interaction. NS: No significant differences, CD: Crohn's disease, ESR: Erythrocyte sedimentation rate, MMP-6: Matrix metalloproteinases-9, SD: Standard deviation

level was correlated with disease severity in UC patients at the baseline, but no relationship was found after both treatments in both diseases (Table 6).

DISCUSSION

To the best search, only two studies in human have explored the hallmarks of melatonin supplementation in IBD. The first was carried out by Rakhimova *et al.,* in 2010, on patients with IBD which revealed a complete ultrastructural recovery of the colonic mucosa when melatonin was added to the standard therapy [24], but data were little informative as to the cellular or molecular mechanism of melatonin in those patients. Overriding the previous findings, Chojnacki *et al.,* in 2011, tracked this pinpoints of melatonin effect but only in UC patients in the remission state of the disease over a 12 months study period, in which 5 mg of melatonin adjuvant therapy was used at bedtime with mesalamine resulting in maintaining remission status [25].

The primary goal of treatment in IBD is to modulate the disease course to enhance quality of life (QOL) and prevent disability while balancing the risks accompanying with therapy [26,27]. To reach this goal, therapy must be directed to achieve resolution of both objective inflammation and clinical symptoms, as well as normalization of QOL (the overarching goal of the current study). This "treat to target" approach requires a clinician to look beyond clinical symptoms and to assess disease activity as objectively as possible. This method enables a composite assessment of the measurable burden of inflammation, the burden of disease on the patient, and the cumulative complications of disease over its course [27].

In the present study, all patients were aged between 18 and 57 years, and both genders were enrolled in the study with a slight predominance of male over female in both CD and UC study groups (67% and 75%), respectively. Several reports from Western countries stated that the prevalence of adult CD and UC is equal or higher among adult female [28-30].

There are many standard instruments for evaluating clinical symptoms in CD such as the CDAI and HBI which are the commonly used tool for assessing disease response to treatment in the clinical trials [19,21]. In the present study, CD patients were presented with a moderate disease activity (total index score 8–16), at the baseline, interestingly, a great percent of reduction the HBI total index score up to remission status (<5) was produced in patients receiving melatonin adjuvant therapy after 8 weeks of treatment compared to the standard treatment (−61.80% vs. −36.48%) ($p<0.001$). Similar improvement in the disease statue was noticed in patients with UC receiving melatonin adjuvant therapy in addition to their standard immunosuppressant and biological targeting therapy to reach the lowest SCAI total index score (which is considered the standard for assessing disease activity in adult UC clinical trials) and marked percent of reduction (−74.48% vs. −46.66%) ($p<0.001$). No matched study was available to interpret this effect but probably could be attributed to the well-known role of melatonin in regulating the circadian rhythm and sleeping during the night [31] since sleep deprivation results in an upregulation of the immune function, which, in turn, stimulates inflammatory cells and increase the risk of infection in patients with IBD [32], or through modulation of primary immune response in the existence of a transient or chronic aggravated immune response, such as septic shock, melatonin might impact a negative regulation could be assumed to act as an anti-inflammatory molecul [7]. The indirect antioxidant effects of melatonin include activation of antioxidative enzymes could promote another explanation [33].

The pattern of inflammation between CD and UC in one arm, and powerful anti-inflammatory effect of standard therapy compared to that of melatonin in the other arm, may produce some conflict effect on ESR levels post-treatment since CD patients in the present study have shown a notable decrease in ESR after standard therapy alone

Table 5: Effect of standard treatment and melatonin adjuvant therapy on ESR and MMP-9 in UC patients

Variable	Study groups in UC		
	Group 3	Group 4	p value
ESR (mm/h)			
Pre-treatment	27.50±16.38	27.70±20.85	0.981^{NS}
Post-treatment	21.00±16.91	12.80±9.91	0.202^{NS}
p value	0.050^{NS}	0.013*	0.151^{aNS}
Percentage change	−23.6%	−53.8%	-
MMP-9 (pg/ml)			
Pre-treatment	1920.00±240.15	1902.40±142.22	0.844^{NS}
Post-treatment	1908.90±208.12	1925.40±131.30	0.834^{NS}
p value	0.878^{NS}	0.758^{NS}	0.739^{aNS}
Percent change	−0.57%	1.20%	-

Data presented as mean±SD. [a]Is p value of interaction. NS: No significant differences ($p>0.05$), (*) significant difference ($p<0.05$). UC: Ulcerative colitis, SD: Standard deviation, ESR: Erythrocyte sedimentation rate, MMP-6: Matrix metalloproteinases-9

Table 6: Correlation between the disease activity indices and inflammatory biomarkers in both diseases

Variables	Study groups			
	Group 1		Group 2	
	r	p value	r	p value
Pre-treatment HBI				
ESR (mm/h)	0.657	0.008 (Sig.)	0.508	0.053
MMP (pg/ml)	−0.066	q	0.006	0.984
Post-treatment HBI				
ESR (mm/h)	0.534	0.040 (Sig.)	0.542	0.037 (Sig.)
MMP (pg/ml)	0.216	0.440	0.045	0.873
Pre-treatment SCCAI				
ESR (mm/h)	0.669	0.034 (Sig.)	0.878	0.001 (Sig.)
MMP (pg/ml)	–0.026	0.943	0.671	0.034 (Sig.)
Post-treatment SCCAI				
ESR (mm/h)	0.455	0.187	0.266	0.458
MMP (pg/ml)	0.594	0.070	−0.440	0.203

r: Pearson correlation coefficient. HBI: Harvey-Bradshaw index, ESR: Erythrocyte sedimentation rate, MMP-6: Matrix metalloproteinases-9

($p<0.05$) and marked decrease after melatonin adjuvant therapy ($p<0.01$), although did not reach the statistical significance between the groups. On the other hand, ESR level in UC patients showed remarkable decrease post-treatment with melatonin supplementation only when compared to the standard therapy alone ($p<0.05$). Accordingly, ESR level was significantly correlated to the improvement in disease activity score in CD patients only post-treatment with melatonin adjuvant.

In IBD, mucosal inflammation leads to collagen deposition, which, in turn, results in the induction of MMPs. The MMP-2 and -9 are transcriptionally unregulated in response to the proinflammatory cytokines or cell-ECM interactions. The MMP-9 mucosal expression and protein levels, as well as serum antigen levels, were significantly higher in UC patients compared to controls, but MMP-9 does not seem to contribute to the severity of the disease. Meanwhile, MMP-2 has shown to be upregulated in pediatric CD [34]. The recently described roles for MMP-9 in IBD include VEGF-A processing, chemokine expression, neutrophil infiltration, generation of antiangiogenic factors, decreased goblet cell differentiation, and prevention of fibrosis [35-39].

In this current study, CD patients presented with slight reduction in MMP-9 level following on melatonin adjuvant therapy compared to standard therapy ($p>0.05$), meanwhile, a conflict results were noticed in patients with UC. Neither the standard treatment that contains the potent anti-TNF-α infliximab nor with melatonin adjuvant therapy,

the MMP-9 level did not reveal significant modulation in the current study, and this is probably due to chronicity of the disease, active phase of both diseases, or short duration of follow-up. No available human histological or clinical data in IBD patients in the active phase to precisely interpret this result, nevertheless, the possible explanation is that in chronic inflammatory diseases such as rheumatoid arthritis and no marked change in the TNF-α level, and hence, in MMPs were noticed after melatonin adjuvant [40] since MMP-9 is upregulated in response to proinflammatory cytokines, then we do not expect to find any speed up modulation accordingly.

Experimentally, melatonin was investigated thoroughly and various experimental studies demonstrated an increase in the matrix-degrading proteases in inflammatory bowel diseases, and the inhibition of MMP activation has been shown to ameliorate experimental colitis, it prevents colitis by regulating MMP-9 and MMP-2 activity and expression. It also alleviates MMP-9 and MMP-3 expression in gastric ulcers through the reduction of activator protein-1 activity. Furthermore, it has been reported that reduced MMP-9 and MMP-2 activities are associated with lower expression of TNF-α [3]. Cuzzocrea *et al.* also showed that melatonin monotherapy significantly reduced the appearance of diarrhea and the reduced body weight in rats induced colitis, the effect is mediated by decreasing the production of TNF-α, reducing the activation of NF-κB, decreasing the expression and activity of MMP-9 and -2, and the antiapoptotic effect of melatonin [41].

The small-scale population of the current study and the melatonin was used in the minimum safe and effective dose to explore its effect subjectively and objectively, also the short duration of the regimen course for patient follow-up gave few limitations to better interpret the effect of melatonin as adjuvant treatment in this active stage of the disease. Nevertheless, the primary goal of the current study was achieved through improvement in the disease activity status in both patients with CD and UC after receiving melatonin adjuvant therapy.

CONCLUSION

Melatonin, through its multiple defense mechanisms against colonic inflammatory processes, produced well-defined positive role to improve the activity status in both patients with CD and UC after receiving melatonin adjuvant in active stage of disease, and notable modulation of disease biomarkers but no clear role on MMP-9 expression, leading to speculate that melatonin adjuvant therapy could be addressed in the future advances in the management of IBD; yet, further, histopathological investigations are warranted.

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CONFLICTS OF INTEREST

The author reported no conflict of interest and no funding was received on this work.

REFERENCES

1. Mozaffari S, Abdollahi M. Melatonin, a promising supplement in inflammatory bowel disease: A comprehensive review of evidences. Curr Pharm Design 2011;17:4372-8.
2. Saniabadi AR, Tanaka T, Ohmori T, Sawada K, Yamamoto T, Hanai H. Treating inflammatory bowel disease by adsorptive leucocytapheresis: A desire to treat without drugs. World J Gastroenterol 2014;20:9699- 715.
3. Jena G, Trivedi PP. A review of the use of melatonin in ulcerative colitis: Experimental evidence and new approaches. Inflamm Bowel Dis 2014;20:553-63.
4. Bubenik GA. Thirty four years since the discovery of gastrointestinal melatonin. J Physiol Pharm 2008;59 Suppl 2:33-51.
5. Jockers R, Maurice P, Boutin JA, Delagrange P. Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? Br J Pharm 2008;154:1182-95.
6. Chen CQ, Fichna J, Bashashati M, Li YY, Storr M. Distribution, function and physiological role of melatonin in the lower gut. World J Gastroenterol 2011;17:3888-98.
7. Carrillo-Vico A, Lardone PJ, Álvarez-Sánchez N, Rodríguez-Rodríguez A, Guerrero JM. Melatonin: Buffering the immune system. Int J Mol Sci 2013;14:8638-83.
8. Ravishankar GA, Ramakrishna A. Serotonin and melatonin: Their functional role in plants. Food, Phytomedicine, and Human Health. Boca Raton: CRC Press; 2016.
9. Tahan G, Gramignoli R, Marongiu F, Aktolga S, Cetinkaya A, Tahan V, *et al*. Melatonin expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic-acid-induced colitis in rats. Dig Dis Sci 2011;56:715-20.
10. Kandil TS, Mousa AA, El-Gendy AA, Abbas AM. The potential therapeutic effect of melatonin in gastro-esophageal reflux disease. BMC Gastroenterol 2010;10:7.
11. Celinski K, Konturek PC, Konturek SJ, Slomka M, Cichoz-Lach H, Brzozowski T, *et al*. Effects of melatonin and tryptophan on healing of gastric and duodenal ulcers with *Helicobacter pylori* infection in humans. J Physiol Pharm 2011;62:521-6.
12. Abbas SH, Abdulridha MK, Najeb AA**.** Potential benefit of curcumin adjuvant therapy to the standard *Helicobacter pylori* eradication therapy in patients with peptic ulcer disease. Asian J Pharm Clin Res 2017;10:313-7.
13. Gopinathan S, Rameela N. Anti-ulcer activity of *Aloe vera* juice and *Aloe vera* and amla fruit combined juice in ethanol induced ulcerated rats. Int J Pharm Pharm Sci 2014;6:190-7.
14. Siah KT, Wong RK, Ho KY. Melatonin for the treatment of irritable bowel syndrome. World J Gastroenterol 2014;20:2492-8.
15. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine 2005;27:189-200.
16. Klupinska G, Wisniewska-Jarosinska M, Harasiuk A, Chojnacki C, Stec-Michalska K, Blasiak J, *et al*. Nocturnal secretion of melatonin in patients with upper digestive tract disorders. J Physiol Pharmacol 2006;57 Suppl 5:41-50.
17. Harbord M, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, *et al*. Third European evidence-based consensus on diagnosis and management of ulcerative Colitis. Part 2: Current management. J Crohn's Colitis 2017;2017:1-24.
18. Gomollón F, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO, *et al*. 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 1: Diagnosis and medical management. J Crohn's Colitis 2017;11:3-25.
19. Peyrin-Biroulet L, Panés J, Sandborn WJ, Vermeire S, Danese S, Feagan BG, *et al.* Defining disease severity in inflammatory bowel diseases: Current and future directions. Clin Gastroenterol Hepatol 2016;14:348-54.
20. National Clinical Guideline C. National Institute for Health and Clinical Excellence: Guidance. Ulcerative Colitis: Management in Adults, Children and Young People. London: Royal College of Physicians (UK); National Clinical Guideline Centre; 2013.
21. Walsh AJ, Bryant RV, Travis SP. Current best practice for disease activity assessment in IBD. Nat Rev Gastroenterol Hepatol 2016;13:567-79.
22. Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR. Acute Phase Reactants and the Concept of Inflammation. Kelley's Textbook of Rheumatology. 9th ed. Ch 57. Philadelphia, PA: Elsevier Saunders; 2013. p. 818-29.
23. Human MMP-9 (Matrix Metalloproteinase 9) ELISA Kit. (Catalog No: E-EL-H1451). 8th ed. China: Elabscience Biotechnology; 2016. Available from: http://www.elabscience.com/index.php/product/view/aid/704.jsp. [Last updated on Aug 2016; Last cited on 2017 Jul 02].
24. Rakhimova O. Use of melatonin in combined treatment for inflammatory bowel diseases. Ter Arkh 2010;82:64-8.
25. Chojnacki C, Wisniewska-Jarosinska M, Walecka-Kapica E, Klupinska G, Jaworek J, Chojnacki J. Evaluation of melatonin effectiveness in the adjuvant treatment of ulcerative colitis. J Physiol Pharm 2011;62:327- 34.
26. Allen PB, Peyrin-Biroulet L. Moving towards disease modification in inflammatory bowel disease therapy. Curr Opin Gastroenterol 2013;29:397-404.
27. Gecse KB, Brandse JF, van Wilpe S, Lowenberg M, Ponsioen C, van

den Brink G, *et al*. Impact of disease location on fecal calprotectin levels in Crohn's disease. Scand J Gastroenterol 2015;50:841-7.
28. Bernstein CN, Wajda A, Svenson LW, MacKenzie A, Koehoorn M, Jackson M, *et al*. The epidemiology of inflammatory bowel disease in Canada: A population-based study. Am J Gastroenterol 2006;101:1559- 68.
29. Kappelman MD, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, *et al.* The prevalence and geographic distribution of crohn's disease and ulcerative colitis in the united states. Clin Gastroenterol Hepatol 2007;5:1424-9.
30. Thia KT, Loftus EV Jr., Sandborn WJ, Yang SK. An update on the epidemiology of inflammatory bowel disease in asia. Am J Gastroenterol 2008;103:3167-82.
31. Halvani A, Mohsenpour F, Nasiriani K. Evaluation of exogenous melatonin administration in improvement of sleep quality in patients with chronic obstructive pulmonary disease. Tanaffos 2013;12:9-15.
32. Ranjbaran Z, Keefer L, Farhadi A, Stepanski E, Sedghi S, Keshavarzian A, *et al.* Impact of sleep disturbances in inflammatory bowel disease. J Gastroenterol Hepatol 2007;22:1748-53.
33. Zhang HM, Zhang Y. Melatonin: A well-documented antioxidant with conditional pro-oxidant actions. J Pineal Res 2014;57:131-46.
34. O'Sullivan S, Gilmer JF, Medina C. Matrix metalloproteinases in inflammatory bowel disease: An update. Mediators Inflamm 2015;2015:19.
35. Heljasvaara R, Nyberg P, Luostarinen J, Parikka M, Heikkilä P, Rehn M, *et al.* Generation of biologically active endostatin fragments from human collagen XVIII by distinct matrix metalloproteases. Exp Cell Res 2005;307:292-304.
36. Deng X, Tolstanova G, Khomenko T, Chen L, Tarnawski A, Szabo S, *et al.* Mesalamine restores angiogenic balance in experimental ulcerative colitis by reducing expression of endostatin and angiostatin: Novel molecular mechanism for therapeutic action of mesalamine. J Pharmacol Exp Ther 2009;331:1071-8.
37. Lee S, Jilani SM, Nikolova GV, Carpizo D, Iruela-Arispe ML. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. J Cell Biol 2005;169:681-91.
38. Pope JL, Bhat AA, Sharma A, Ahmad R, Krishnan M, Washington MK, *et al.* Claudin-1 regulates intestinal epithelial homeostasis through the modulation of notch-signalling. Gut 2014;63:622-34.
39. Bailey JR, Bland PW, Tarlton JF, Peters I, Moorghen M, Sylvester PA, *et al*. IL-13 promotes collagen accumulation in Crohn's disease fibrosis by down-regulation of fibroblast MMP synthesis: A role for innate lymphoid cells? PLoS One 2012;7:e52332.
40. Forrest CM, Mackay GM, Stoy N, Stone TW, Darlington LG. Inflammatory status and kynurenine metabolism in rheumatoid arthritis treated with melatonin. Br J Clin Pharm 2007;64:517-26.
41. Esposito E, Mazzon E, Riccardi L, Caminiti R, Meli R, Cuzzocrea S. Matrix metalloproteinase-9 and metalloproteinase-2 activity and expression is reduced by melatonin during experimental colitis. J Pineal Res 2008;45:166-73.