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Efficacy of melatonin in the treatment of endometriosis: A phase II, randomized, double-blind, placebo-controlled trial

André Schwertner [a,1], Claudia C. Conceição dos Santos [a,b,1], Gislene Dalferth Costa [a,b], Alícia Deitos [a,b], Andressa de Souza [b], Izabel Cristina Custodio de Souza [a,b], Iraci L.S. Torres [b,c], João Sabino L. da Cunha Filho [b], Wolnei Caumo [a,b,c,d,*]

[a] Laboratory of Pain & Neuromodulation at Hospital de Clínicas de Porto Alegre (HCPA)/Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[b] Post Graduate Program in Medical Sciences, School of Medicine, UFRGS, Porto Alegre, Brazil
[c] Pharmacology Department, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, Brazil
[d] Pain and Palliative Care Service at the HCPA, UFRGS, Porto Alegre, Brazil

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ABSTRACT

Endometriosis-associated chronic pelvic pain (EACPP) presents with an intense inflammatory reaction. Melatonin has emerged as an important analgesic, antioxidant, and antiinflammatory agent. This trial investigates the effects of melatonin compared with a placebo on EACPP, brain-derived neurotrophic factor (BDNF) level, and sleep quality. Forty females, aged 18 to 45 years, were randomized into the placebo (n = 20) or melatonin (10 mg) (n = 20) treatment groups for a period of 8 weeks. There was a significant interaction (time vs group) regarding the main outcomes of the pain scores as indexed by the visual analogue scale on daily pain, dysmenorrhea, dysuria, and dyschezia (analysis of variance, $P < 0.01$ for all analyses). Post hoc analysis showed that compared with placebo, the treatment reduced daily pain scores by 39.80% (95% confidence interval [CI] 12.88–43.01%) and dysmenorrhea by 38.01% (95% CI 15.96–49.15%). Melatonin improved sleep quality, reduced the risk of using an analgesic by 80%, and reduced BNDF levels independently of its effect on pain. This study provides additional evidence regarding the analgesic effects of melatonin on EACPP and melatonin's ability to improve sleep quality. Additionally, the study revealed that melatonin modulates the secretion of BDNF and pain through distinct mechanisms.

1. Introduction

Chronic pelvic pain (CPP) is defined as a nonmalignant pain perceived in the structures related to the pelvis that is constant or recurring over a period of 6 months [21]. A population study demonstrated a 3.8% occurrence rate [67]; however, in infertility populations, this rate can be as high as 40% [50].

Endometriosis is strongly associated with CPP. Endometriosis lesions produce pain by compressing or infiltrating the nerves near the lesions [54]. The presence of nerve growth factors in lesions is correlated with hyperalgesia [54]. Nerve growth factor is also responsible for the growth of sympathetic and sensory neurons of ectopic endometrial growths [54]. C-fiber nociceptors these neurons are activated by noxious events, such as inflammation; not only do they convey information to the central nervous system (afferent function), but they can release substance P, calcitonin gene-related peptide, tachykinins, nitric oxide, and other factors into the local environment (efferent functions). Once activated, C fibers can become sensitized; efferent activity also increases the local vascular permeability and the "neurogenic inflammation" [55].

Although the full pathophysiology of CPP remains unknown, cumulative evidence suggests that peripheral and central sensitization result in an amplification of sensory impulses [63]. It has been suggested that estrogen increases brain-derived neurotrophic factor (BDNF) during the estrous cycle [7], and BDNF has received attention as a neuromediator of hyperalgesia and spinal central sensitization in pain states [17,28]. In an experimental study, BDNF enhanced the potentiation of *N*-methyl-D-aspartate receptors in dorsal spinal horn neurons, increasing the neurons' excitability [25]. In another experiment, the neurotoxin mycotoxin 3-nitropropionic acid also increased the BDNF and neuronal excitability [57]; however, melatonin reversed this effect. These results seem mixed, as in intact cells, others have found that melatonin increases the BDNF level [35,45]. Thus, we postulate that in chronic

* Corresponding author. Address: Laboratory of Pain & Neuromodulation, Hospital de Clínicas de Porto Alegre at UFRGS, Rua Ramiro Barcelos 2350, CEP 90035-003 Bairro, Rio Branco, Porto Alegre, RS, Brazil. Tel.: +55 51 3359 8083; fax: +55 51 3359 8083.
E-mail address: caumo@cpovo.net (W. Caumo).
[1] These authors are both first authors.

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pain, melatonin may reduce the activity of the neurotrophic factors, particularly BDNF, that play an important role in the pathogenesis of chronic pain.

Despite therapeutic advances, the options available for the treatment of endometriosis have a limited impact on the course of the disease and produce unsatisfactory results while causing poorly tolerated adverse effects [8]. It is important to investigate other therapeutic options, such as melatonin, which has been shown to have a beneficial effect in experimental models of endometriosis [65]. Mounting evidence indicates that melatonin should be tested in this setting, considering its analgesic, antioxidant [27,32], and antiinflammatory effects. In fact, the antinociceptive effect of melatonin has been demonstrated in animal models of acute pain [20,39], inflammatory pain [5,6,19], and neuropathic pain [60]. In the few studies conducted with humans, doses of 3–10 mg of melatonin have been used [2,11,12,66].

Overall, these data prompted us to investigate whether melatonin can be used as a therapeutic agent in endometriosis. In this context, we tested the hypothesis that melatonin would be more effective than a placebo for the treatment of endometriosis-associated CPP (EACPP). We also tested whether melatonin would change the levels of BDNF and whether it would be more effective than a placebo in improving sleep quality.

2. Methods

The Methods and Results sections are reported according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines [52].

2.1. Design overview, setting, and participants

All patients provided written informed consent before participating in this randomized, double-blind, 2-group parallel, clinical trial, which was approved by the Research Ethics Committee at the Hospital de Clínicas de Porto Alegre (Institutional Review Board IRB 0000921) in accordance with the Declaration of Helsinki (Resolution 196/96 of the National Health Council). We recruited 40 patients complaining of pelvic pain, who were between 18 and 45 years old, from the gynecology outpatient clinic at the Hospital de Clínicas de Porto Alegre and by newspaper publicity. We defined chronic pelvic pain and/or dyspareunia as a moderate-to-severe pain intensity lasting for more than 6 months [22], eliciting pain scores on a categorical scale (0 to 10) equal to or higher than 4 and requiring regular analgesic use [46]. All patients had an endometriosis diagnosis confirmed by laparoscopic surgery in a pelvic pain investigation by the same investigator (J.S.L.C.). The endometriosis stages included the following: stage 1 (only a few endometrial implants, mostly found in the cul-de-sac and pelvic area); stage 2 (at least one of the ovaries had endometriosis); stage 3 (usually both ovaries, as well as the uterus, had endometriosis); and stage 4 (endometriosis that was prominent in the abdominal cavity and may affect many surrounding organs). Nongynecologic causes of pelvic pain were excluded in all patients using history, physical examination, and laboratory examinations when appropriate. We excluded patients with diagnosed malignancies, uterine myomas, ovarian cysts, inflammatory pelvic disease, and pregnancy. Patients with a history of neurologic or oncologic disease, ischemic heart disease, kidney or hepatic insufficiency, or a regular intake of antidepressants or anticonvulsants that could not be discontinued at least 15 days before the start of the study were not included. Additionally, no patients were included who had a history of alcohol or substance abuse in the past 6 months or who were undergoing hormonal therapy or had irregular cycles. All of these conditions could interfere with marker levels or disease symptom measurements.

2.2. Sample size justification

The number of patients in each study group was determined by previous clinical trials [23]. An a priori estimate indicated that a total sample size of 36 patients divided into 2 balanced treatment groups (n = 18) was needed to detect a 1.3-cm reduction (average SD 1.2 cm) in pain intensity associated with melatonin or placebo at power and α levels of 0.8 and 0.01, respectively [16,53]; such a reduction would be clinically relevant and comparable to other pharmacological interventions. To account for multiple outcomes and attrition, we increased the sample size to 40 patients, 20 per group. For these calculations, we assumed that this remission was clinically relevant.

2.3. Randomization and masking

We used a fixed block size of 4 to ensure that equal numbers of participants were randomized into the 2 groups. Before the recruitment phase, envelopes containing the protocol materials were prepared. Each envelope was sealed and numbered sequentially and contained an allocated treatment. After the participant agreed to participate in the trial, the envelope in the sequence was opened by the nurse who administered the medications. During the entire protocol timeline, 2 investigators who were not involved in patient evaluations were responsible for the blinding and randomization procedures. Other individuals who were involved in patient care were unaware of the treatment group to which the patients belonged.

2.4. Interventions

All patients began the treatment at the onset of the menstrual cycle. Over an 8-week period (56 days), the following oral medications were taken at bedtime by the 2 groups: 10-mg melatonin tablets (Sigma Chemical, Munich, Germany, provided batch-by-batch certificates of analysis authenticating the purity of each batch) or a placebo with identical characteristics. The capsules were manufactured in such a way that the placebo and active treatment appeared to be identical. To measure adherence to medication use, we employed the following strategies: (1) a researcher counted the number of tablets consumed each week during the study period; (2) the patients were asked to record a diary entry if they failed to use the medication; and (3) eligible patients were strongly encouraged to remain on the medication throughout the 8 weeks, during which time they were assessed weekly in a visit to the clinical center. Regardless of their decision to continue or discontinue medication at this stage, the patients continued to be assessed during the study period.

2.5. Supplementary analgesic use

All of the patients were permitted to use supplementary analgesic medication (acetaminophen, ibuprofen, codeine, or tramadol) to relieve their pain if necessary. Patients were allowed to take 750 mg of acetaminophen up to 4 times per day (QID) and 200 mg of ibuprofen at maximum QID as a rescue analgesic. If their pain persisted, patients were permitted to use 60 mg of codeine up to QID or tramadol 3 times per day. The patients were asked to record their analgesic intake during the treatment period in their pain diaries, and these diaries were reviewed during each treatment section. The total analgesic dose taken during treatment was considered for the analysis.

2.6. Instruments and assessments

All of the psychological tests used in this study had been validated for the Brazilian population [31,33]. Two independent

medical examiners who were blind to the group assignments were trained to administer the pain scales and to conduct the psychological tests. The baseline depressive symptoms of the patients were assessed using the Hamilton Depression Scale [31], and sleep quality was assessed using the Pittsburgh Sleep Quality Index [10]. Psychiatric disorders were evaluated with the Structured Clinical Interview for DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition) Axis I Disorders [40], and anxiety was measured using the refined version of the Rash analysis of the State-Trait Anxiety Inventory [33]. Demographic data and medical comorbidities were assessed using a standardized questionnaire; patients were asked about any changes that occurred during treatment, such as changes in mood, sleepiness, dizziness, headaches, or allergic reactions.

2.7. Outcomes

The primary outcome was pain, as assessed by the pain score diaries (global pain in the last 24 hours, painful menstrual periods [dysmenorrhea], or discomfort during intercourse [dyspareunia]), the amount of analgesics used weekly throughout the treatment period, and the level of BDNF. Secondary outcomes were discomfort during urination (dysuria) or defecation (dyschezia), and sleep quality. The outcomes are described below.

2.7.1. Assessment of pain and sleep

(a) Pain intensity was measured by a 10-cm visual analogue scale (VAS). The VAS scores ranged from no pain (0) to worst possible pain (10 cm). The time of the worst pain during the last 24 hours was recorded daily in the patients' diaries. They were asked to answer the following questions using the pain VAS: (1) Considering your chronic pelvic pain in normal period – how intense was your worst pain during the last 24 hours?; (2) Considering the changes in pain at the onset of menstrual bleeding – How intense was your pain during your menstrual period?; (3) How intense was your pain during intercourse?; (4) How intense was your pain during defecation?; and (5) How intense was your pain during urination? To improve patient compliance, an evaluator checked their pain records weekly. Pain scores during menstruation were assessed for 7 days at a time.

(b) Diary entries recording analgesic intake (acetaminophen, nonsteroidal antiinflammatory drugs [NSAIDs], or opioids) were reviewed during each treatment section. The total analgesic dose taken during treatment was considered for analysis.

(c) Sleep quality during the study period was assessed daily by the 10-cm visual analogue sleep quality scale (VASQS). VASQS scores ranged from worst possible (0) to best possible (10 cm) sleep, and using the VASQS, the patients answered the following question in their sleep diaries: (a) How well did you sleep last night?

(d) Laboratory outcomes included serum levels of BDNF. Samples of blood were collected at 2 time points: at baseline and at the end of treatment. The blood samples were centrifuged in plastic tubes for 10 minutes at 4500×*g* at 4°C, and serum was stored at −80°C for hormone assay. Serum BDNF was determined by enzyme-linked immunosorbent assay (ELISA) using a ChemiKine BDNF Sandwich ELISA Kit, CYT306 (Chemicon/Millipore, Billerica, MA, USA). The lower detection limit of the kit is 7.8 pg/mL for BDNF. We analyzed the effects of age in our model.

2.8. Statistical analysis

Figs. 1 and 3 *T*-tests for independent samples were used to analyze the continuous variables, and the categorical variables were

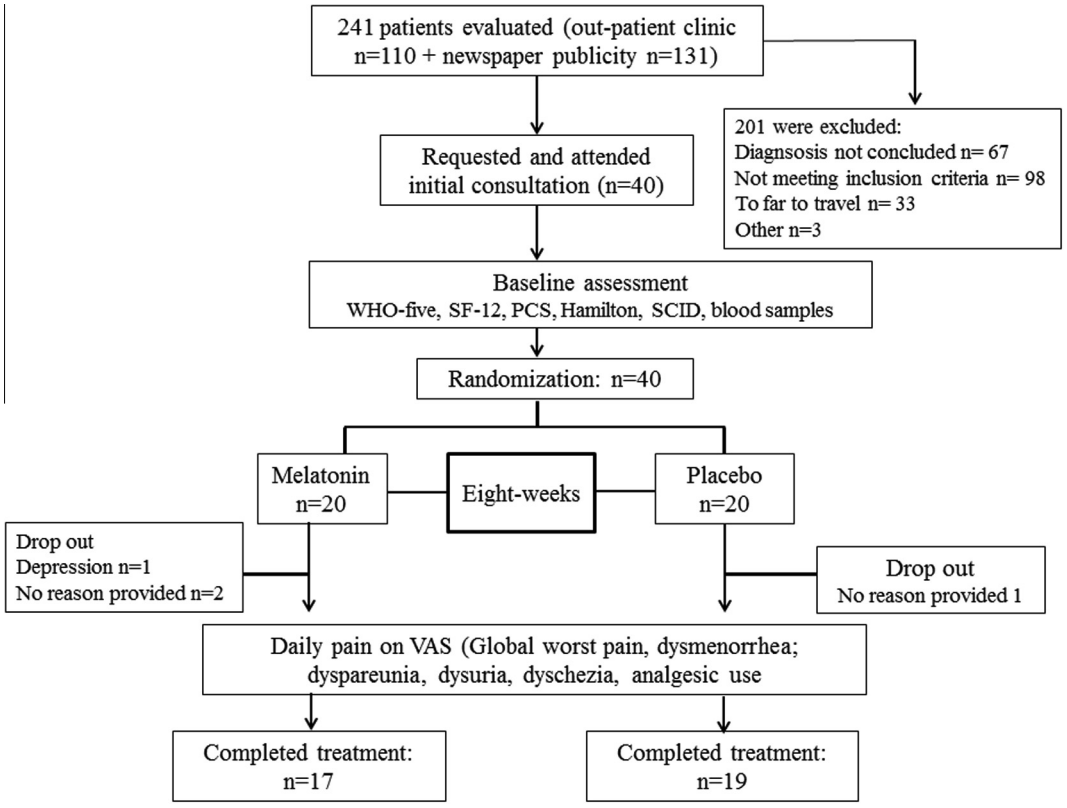

Fig. 1. Flow chart showing recruitment and progress through the study. WHO, World Health Organization; SF-12, Short-Form 12 health survey; PSQ, Pittsburgh Sleep Questionnaire; SCID, Structured Clinical Interview for DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition); VAS, visual analogue scale.

assessed by χ^2 or Fisher's exact tests. We averaged the daily values recorded in the pain and sleep diary to generate one value for each of the 8 weeks of treatment. After first checking the assumptions of normality for the outcome measures (VAS pain scores and VASQS sleep quality scores) using skewness and kurtosis tests, we conducted a group analysis by running a mixed analysis of variance model in which the independent variables were time, experimental group (melatonin and placebo), the interaction between time and experimental group, and the subject identification. If appropriate, we then performed Bonferroni test for post hoc multiple comparisons to identify the differences between the groups at each time point and used a paired *t*-test to assess the effects of the variables on each experimental group. Stepwise multiple linear regression analysis was conducted, with the VAS pain scores as the dependent variable and the experimental group (melatonin and placebo) and sleep quality the previous night as independent variables.

We also calculated adjusted mean differences, which were defined as the relative changes in the melatonin group compared to the placebo group. This measurement was used to describe the melatonin treatment efficacy, which was calculated as the adjusted mean difference divided by the adjusted mean placebo group and expressed as a percentage (%). The 95% confidence intervals (CI) and associated *P*-values were also calculated. We considered all of the randomized patients as part of the analysis, using the intention-to-treat method, with the last observation carried forward. The data were analyzed using SPSS version 18.0 (SPSS, Chicago, IL).

3. Results

3.1. Patient characteristics

The clinical and demographic characteristics of the patients are shown in Table 1. Twenty patients were allocated to the placebo group, and 20 were allocated to the melatonin group. Thirty-six patients completed the study; 3 patients in the melatonin group and one in the placebo group withdrew due to treatment inefficacy. Baseline characteristics were similar across the melatonin and placebo groups (all *P* values >0.05) (Table 1). We did not observe serious or moderate side effects from the treatment.

3.2. Analysis of the main outcome: efficacy with regard to pain scores (highest pain level during the last 24 hours and dysmenorrhea), analgesic use, and BDNF

The melatonin group had significantly lower pain VAS scores ($P < 0.001$) than the placebo-treated group (Table 2), and the interaction between time and treatment group was significant ($P = 0.02$) (Fig. 2A). The melatonin group, compared to the placebo group, demonstrated a mean pain reduction of 39.30% (Table 2). The melatonin group had significantly lower pain scores ($P < 0.001$) than the placebo-treated group during menstruation (Table 2), and again, the interaction between time and treatment group was significant ($P \leqslant 0.001$) for these VAS scores (Fig. 2B).

Analgesic use during the treatment period occurred in 42.2% of patients in the placebo group and in 22.9% of patients in the melatonin group. The relative risk for using analgesics at least 3 times a week during the 8 weeks of treatment was 1.80 (95% CI 1.61–2.08); that is, the placebo-treated group was 80% more likely to require additional analgesics. Of the analgesics used in the placebo group, acetaminophen was used by 66.7% of the patients, codeine or tramadol by 60%, and NSAIDs by 60% (the same patient may have used more than one analgesic). In the melatonin group,

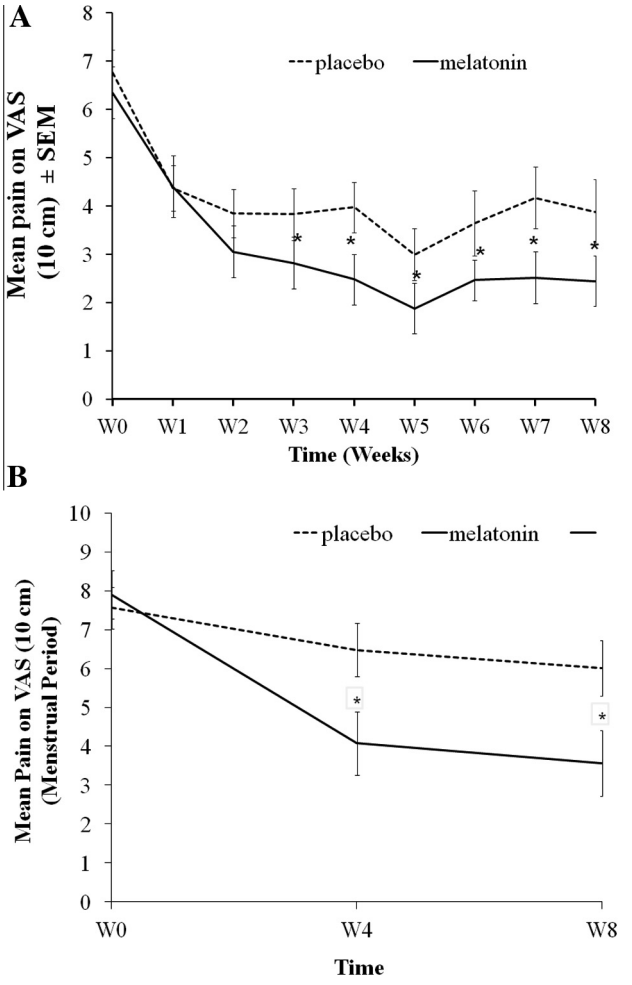

Fig. 2. (A) Weekly mean pain levels (as assessed by visual analogue scale [VAS]) at baseline week (W) zero to W8, in the 2 experimental groups for the question: Considering your chronic pelvic pain in normal period – how intense was your worst pain during the last 24 hours? (B) Considering the changes in pain at the onset of menstrual bleeding – How intense was your pain during your menstrual period? The error bars indicate the standard error of the mean. Asterisks (*) positioned above the bars indicate significant differences ($P < 0.01$) at those time points between the placebo and melatonin groups. All comparisons were performed by a mixed analysis of variance model, followed by the Bonferroni correction for post hoc multiple comparisons.

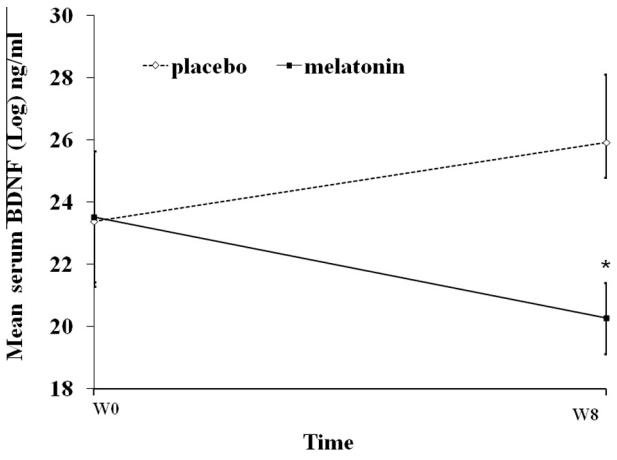

Fig. 3. Mean serum brain-derived neurotrophic factor (BDNF) at baseline week (W) zero and W8, in the 2 experimental groups. The error bars indicate the SEM. Asterisks (*) positioned above the symbols indicate significant differences ($P < 0.01$) at those time points between the placebo and melatonin groups compared using the *t*-test for independent samples.

Table 1
Characteristics of the study sample. Values are given as the mean (SD) or frequency (n = 40).

	Placebo (n = 20)	Melatonin (n = 20)	*P*-value
Age (years)	37.63 (±5.5)	36.76 (±6.4)	.86
Education (years)	10.7 (±3.51)	11.0 (±1.87)	.72
Smoking, n (%)	1 (5.0%)	5 (25.0%)	.08
Clinical comorbidity	7 (35.0%)	6 (30.0%)	.94
Hypertension	0 (0.0%)	3 (15.0%)	.06
Hypothyroidism	2 (10.0%)	1 (5.0%)	.61
Asthma	2 (10.0%)	0 (0.0%)	.17
Other	3 (15.0%)	2 (10.0%)	.49
Endometriosis stage			
I	3 (15%)	2 (10%)	
II	3 (15%)	4 (20%)	
III	9 (45%)	7 (35%)	
IV	5(25%)	7 (35%)	.63
Global pain on visual analogue scale	6.89 (±2.1)	6.46 (±2.6)	.73
Menstrual pain on visual analogue scale	8.00 (±2.0)	7.32 (±2.5)	.30
Pittsburgh Sleep Questionnaire	15.6 (±7.6)	19.0 (±5.9)	.15
Hamilton Depression Scale	18.4 (±8.4)	16.3 (±10.7)	.51
Psychiatric disease (SCID-I)	11 (55.0%)	12 (60.0%)	.34
Depression	7 (35.0%)	6 (30.0%)	.94
Anxiety	11 (55.0%)	9 (45.0%)	.78
Daily use of analgesics	16 (80.0%)	15 (75.0%)	.73
Acetaminophen/dipirone	12 (60.0%)	7 (35.0%)	.25
NSAID	9 (45.0%)	10 (50.0%)	.37
Opioid	2 (10.0%)	3 (15.0%)	.49

SCID-I, Structured Clinical Interview for DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition) Axis I disorders; NSAID, nonsteroidal antiinflammatory drug.

33.3% used acetaminophen, 40% used tramadol or codeine, and 35% used NSAIDs. There was a significant reduction in the analgesic doses for patients receiving melatonin treatment compared to those receiving placebo ($P < 0.01$).

One important issue is whether the BDNF level reduction is secondary to pain improvement or whether it is a primary effect of the intervention. To address this important issue, we conducted an additional regression model in which we controlled BDNF-level changes for cumulative pain scores during the treatment period. The adjusted mean BDNF level for the placebo group was 25.64 (9.65) vs 20.46 (4.93) for the melatonin group, with a mean difference of 5.94 (95% CI 2.07–9.82); this difference was significant at the $P < 0.001$ level. This model revealed that the effect of the pain score was not statistically significant. However, the interaction between group and pain VAS scores was significant ($P < 0.001$), suggesting that the variability in the pain VAS score is dependent on the effects of the treatment group on the main outcome (BDNF levels). Additionally, when we performed a stratified analysis by treatment group, the effect of pain was not statistically significant in the melatonin group, but was significant in the placebo group (Table 3). This result suggests that the effect of treatment on the BDNF level is not dependent on the pain level.

3.3. Secondary outcomes: evacuation pain, dysuria, and sleep quality

There was a significant interaction between time and treatment group ($P < 0.01$) for pain VAS scores during evacuation and dysuria. The melatonin group recorded significantly lower pain scores (for both outcomes $P < 0.01$) compared to the placebo group (Table 2).

There was a significant interaction between time and treatment group ($P < 0.001$) for the previous night's sleep quality VASQS scores. Patients in the melatonin group reported a significantly better sleep quality ($P < 0.001$) than the placebo group (Table 2). When compared with the placebo, melatonin produced a mean improvement of 42% in how patients felt when they awoke (Table 2).

4. Discussion

This study demonstrated that melatonin produced a reduction in EACPP compared to placebo that was not only statistically significant but may also be clinically relevant. In addition, this study showed that melatonin treatment was associated with improved sleep quality and reduced BDNF levels. This finding suggests that melatonin has a direct effect on pain pathways or on the levels of signaling chemicals that regulate pain. Furthermore, the present findings showed that the effect of melatonin on BDNF level is independent of its effect on pain.

These findings concur with the results of previous studies in animals in which melatonin caused the regression and atrophy of endometriotic lesions [29]. However, to the best of our knowledge, this is the first investigation that extends this literature to provide additional evidence of the impact of melatonin on EACPP. Melatonin

Table 2
Treatment effect on the outcomes during the treatment period (8 weeks) (n = 40).

Pain reported on visual analogue scale					
	Treatment	Adjusted mean (SD)	Adjusted mean difference (95% CI) [a]	Relative change % (95% CI) [b]	*P*-value
Primary outcomes					
Worst pain during the last 24 hours (daily)	Placebo (n = 20)	4.58 (1.46)			
	Melatonin (n = 20)	2.78 (1.35)	1.80 (0.59-1.97)	39.30 (12.88-43.01)	< 0.001
Pain during menstrual period (dysmenorrhea)	Placebo (n = 20)	6.84 (2.38)			
	Melatonin (n = 20)	4.24 (2.61)	2.6 (0.38-1.71)	38.01 (15.96-49.15)	< 0.001
Pain during intercourse	Placebo (n = 20)	6.08 (1.42)			
	Melatonin (n = 20)	4.68 (1.51)	1.40 (0.42-1.49)	23.02 (6.90-24.50)	< 0.001
Secondary outcomes					
Pain during evacuation	Placebo (n = 20)	6.30 (1.64)			
	Melatonin (n = 20)	4.12 (0.97)	2.18 (1.25-2.30)	34.60 (19.84-36.50)	< 0.0001
Pain during urination	Placebo (n = 20)	6.33 (1.31)			
	Melatonin (n = 20)	5.35 (0.69)	1.13 (0.41-1.75)	27.65 (6.47-133.00)	<0.001
How well did you sleep last night – on visual analogue sleep quality scale (VASQS)	Placebo (n = 20)	4.98 (1.51)			
	Melatonin (n = 20)	6.08 (1.42)	1.1 (0.11-1.39)	22.08 (2.20-27.91)	< 0.02

CI, confidence interval.
[a] Mixed analysis of variance model. Mean difference groups.
[b] Relative change = adjusted mean difference/adjusted placebo mean × 100%.

Table 3
Multivariate linear regression of the pain reported compared with BDNF level, treatment group and daily pain VAS scores (n = 40).

Parameter	β	T	P-value	95% CI
Dependent variable: serum BDNF after 8 weeks of treatment				
Cumulative worst pain score on VAS dairy (mean of 8 weeks)	−0.07	−0.09	0.92	−1.54 to 1.40
Placebo ∗ (cumulative pain score on VAS dairy) vs Melatonin ∗ (cumulative pain score on VAS dairy)	2.60	3.14	<0.001	0.90 to 4.33
Interaction	20.46	10.69	<0.001	16.55 to 24
Cumulative pain score on VAS dairy ∗ (Placebo)	2.23	2.33	<0.01	0.25 to 5.02
Cumulative pain score on VAS dairy ∗ (Melatonin)	−0.16	−0.26	0.79	−1.59 to 1.18

BDNF, brain-derived neurotrophic factor; VAS, visual analogue scale; CI, confidence interval.
Linear regression model – Adjusted R^2 = 0.24.

was observed to have a clinically relevant effect on all parameters used to assess pain, including a reduction in daily pain, dysmenorrhea, dyspareunia, dysuria, and dyschezia (Table 2), as well an 80% reduction in analgesic use. Overall, the magnitude of the effect on reported pain was >35%. This result is important because a placebo effect has been well documented in studies of pain control [59]. For example, Koninckx et al. [36] reported a 30% reduction in pain severity associated with placebo, even in women with deep endometriosis. Novel treatments for the symptoms of endometriosis should therefore demonstrate superiority relative to placebo to verify their efficacy.

Additionally, the present study corroborates evidence from previous randomized clinical trials in which melatonin performed substantially better than a placebo in treating fibromyalgia [3,14] and acute postoperative pain [30,31]. This finding has biological plausibility because the antinociceptive effect of melatonin is known to involve the activation of supraspinal sites and the inhibition of "spinal windup" [44]. In addition, experimental evidence suggests that the analgesic effects of melatonin are mediated by opioids [38] and by gamma-aminobutyric acid ([GABA]ergic) systems [64]. Moreover, melatonin produces marked antiinflammatory effects on peripheral sites by inhibiting the release of proinflammatory cytokines [15]. Additionally, its effect on EACPP may be explained by diverse mechanisms, including hormonal pathways, because it is known that endometriosis is an estrogen-dependent chronic inflammatory gynecological disease. Thus, melatonin may, at least in part, decrease the luteinizing hormone surge, thereby blocking ovulation and progression into the luteal phase, and increase progesterone without affecting follicle-stimulating hormone or estrogen levels [41]. Melatonin also inhibits steroidogenesis by altering cyclic adenosine monophosphate levels through direct action on the theca or granulosa cells of the follicles [9,56]. Additionally, the treatment of rats with melatonin resulted in reduced plasma levels of luteinizing hormone and 17 beta-estradiol and promoted differential regulation of the estrogen, progesterone, and androgen receptors in the reproductive tissues [13].

A previous study found that estradiol-dependent estrogen receptor (ER) activation was prevented in cells by treatment with melatonin. Neither the nuclear localization of the ER nor the binding of estradiol to the ER is affected by melatonin [49]. Thus, it is possible that the effect of estrogen may occur through 2 distinct ER subtypes, ERα and ERβ, which are widely distributed in the central nervous system, in astrocytes and in interneurons. Astrocytes enhance the synthesis of astroglial-derived neurotrophic factors and inflammatory mediators, such as cytokines and cycloxygenase-2, which, at least in part, maintain the maladaptive plasticity implicated in EACPP. The inflammatory response involves the hormonal and neuronal mechanisms by which the brain regulates the function of the immune system and cytokines, allowing the immune system to regulate the brain. Although it has been reported that BDNF is involved in inflammatory reactions [34,58] and that its production is increased in response to proinflammatory cytokines, our findings support the notion that BDNF levels may constitute a neurobiological mechanism underlying EACPP. This hypothesis is supported by our finding that the BDNF level was linked with the pain level in the placebo group but not in the melatonin group [37,42]. Thus, the melatonin-induced reduction in this neurotrophin may be explained by other mechanisms; for example, an antiinflammatory mechanism, or perhaps, an interaction with gonadal hormones could be involved.

The effect of melatonin on the BDNF level can be explained by estrogen levels, which regulate the increase in BDNF mRNA in areas associated with nociceptive sensory processing, such as the hippocampus, cerebral cortex, and spinal cord [4]. The relationship between BDNF level and pain may indirectly confirm the role of the regulatory effect of estrogen on the sensitization of the central nervous system centers associated with nociceptive sensory processing. Furthermore, estrogen regulates opioid receptor density in several pain-related areas (periaqueductal gray, parabrachial nucleus); the density of opioid receptors is significantly lower in female rats during proestrus (higher plasma estrogen) compared with the diestrus and metestrus phases [18].

Because BDNF levels can serve as a molecular "sensor" of the global levels of neuronal activity, it has been suggested that the induction of BDNF expression in response to increases in the level of neuronal activity may dampen cortical excitability by promoting the development and/or strengthening of inhibitory synapses in local circuits [26,51]. Overall, these findings show that the melatonin effect may interrupt a response associated with a maladaptive neuroplasticity process orchestrated by neuronal, endocrinal, and immune mechanisms that can amplify sensory pain signals to the neural pain matrix [24]. Macrophage-derived productions, such as the production of tumor necrosis factor, interleukin (IL)-1β, and IL-12, integrate this cascade of responses [1].

Another possible explanation for our findings is the involvement of melatonin in regulating circadian rhythms, which would make melatonin a potentially valuable means for targeting the pathophysiologic mechanisms underlying EACPP. Although melatonin is approved as a sleep aid, it also has a variety of other beneficial effects that may account for its potential role in the treatment of EACPP. Obviously, pain relief is a major goal, but the additional treatment of restless sleep and sleep disturbances may lead to a further decrease in pain [30]. However, in recent randomized clinical trials, we demonstrated that melatonin's effect on pain is independent of improvements in sleep quality [62]. It had been demonstrated that the antinociceptive effects of melatonin involve effects [38] on the gamma-aminobutyric acid and opioid systems [64]. Moreover, melatonin produces marked antiinflammatory effects on peripheral sites by inhibiting the release of proinflammatory cytokines [43].

The strengths of the study include the comparison of an active treatment vs a placebo in a double-blind design, and the use of multiple efficacy and safety measures based on previous trial

experience. Despite the knowledge of a substantial placebo effect, there remains a scarcity of placebo-controlled studies of medications for the treatment of endometriosis pain [47,48]. This study therefore represents an important contribution to evidence-based prescribing. We conducted this trial according to CONSORT guidelines, and given that we used the Delphi List (a list of criteria for the quality assessment of randomized controlled trials), our trial can be considered to be of strong quality because all 8 items in this scale can be positively scored in our randomized controlled trial [61]. Although the homogeneity of this study population is methodologically advantageous, the issue of external validity arises. Thus, additional research with a larger number of patients is needed to more widely assess the potential benefits of melatonin in various clinical settings, and future studies are required before definitive conclusions regarding melatonin and pain treatment can be made. Several issues concerning the design of our study must be addressed. First, a potential limitation is the short treatment duration, as it would have been difficult to justify a prolonged placebo treatment period in patients experiencing chronic pain. Second, the influence of pelvic floor musculature in pelvic pain that is usually assessed by digital palpation during pelvic examinations was not directly measured. Instead, it was assessed indirectly using pain scores associated with various events (ie, pain during urination, sexual intercourse, and defecation). Third, we did not assess changes in menses, which could have been altered due to melatonin effect on ovarian steroidogenesis. Finally, although several strategies were used to prevent patients and evaluator team from unblinding, formal assessment for awareness of the allocation (either active or placebo) was not performed. However, our objective surrogates less prone to bias (ie, serum BDNF, analgesics requirements) were consistent with pain scores, hence, unblinding is unlikely to have influenced the direction of our conclusions.

In conclusion, in this 8-week, randomized, double-blind, placebo-controlled study, the oral consumption of 10 mg/day of melatonin was associated with significant improvements in EACPP and other efficacy measures. Melatonin reduced pain scores, lowered analgesic use, and improved sleep quality. Our results also suggested that melatonin modulates the secretion of BDNF by a mechanism that is distinct from the one underlying its analgesic effect. Overall, melatonin may represent an effective and well-tolerated treatment for the painful symptoms of endometriosis.

Conflict of interest statement

The authors declare that there are no financial or other relationships that might lead to conflicts of interest in any of the following arrangements: financial relationship to the work, employees of a company, consultants for a company, stockholders of the company, members of a speakers' bureau, or any other form of financial support.

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