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Research Article

Clinical Investigation of Protective Effects of Melatonin on Patients with Acute Ischemic Stroke

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ABSTRACT

Background: Stroke is one of the leading causes of annual mortality and disability for many individuals worldwide. Ischemic stroke has a high incidence and mortality rate, which significantly affects the quality of life and places an overwhelming mental and financial burden on the patients' families. Melatonin has a neuroprotective effect on patients with acute ischemic stroke. This study aimed to develop the employment of melatonin on clinical features of acute ischemic stroke.

Methods: This double-blind, placebo-controlled clinical trial was conducted on 70 patients with acute ischemic stroke not eligible for reperfusion therapy who were admitted to Bu-Ali Hospital. The consent form was taken, and all of the patients received routine management. Participants were divided into two groups. The 35 patients received 10 mg of melatonin once daily for five days, and others received 10 mg of placebo. National Institute of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) scores were recorded for all patients before treatment and after on days 5, 30, and 90.

Results: The 70 patients included in this study were based on inclusion criteria. The severity of stroke and the functional status of patients were compared in both groups. The melatonin group showed a significant reduction in the NIHSS score from day five up to day thirty compared to the placebo group ($P = 0.001$). There was no difference in the mRS score between the two groups in this study ($P > 0.05$). The relative frequency of the adverse event of sleepiness in patients receiving melatonin was significantly higher than in patients receiving placebo ($P = 0.022$).

Conclusion: Patients who receive melatonin early after stroke have better improvement in post-stroke recovery and disabilities. These findings verify the results of other studies.

The authors declare no conflicts of interest.
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Introduction

Stroke is one of the leading causes of annual mortality and disability for many individuals worldwide [1, 2]. It affects the lives of millions of patients and imposes a heavy financial burden on society [3, 4]. Ischemic stroke is an acute cerebrovascular disease caused by various reasons that lead to the interruption of blood flow in the cerebral artery and ischemic necrosis of cerebral tissue, leading to loss of neural function [5, 6]. The incidence and mortality rate of ischemic stroke are high [7, 8], which significantly affects the quality of life and places an overwhelming mental and financial burden on the patients' families [9]. At the time being, therapeutic options for ischemic stroke are still limited, and the only drug authorized by the FDA is recombinant tissue plasminogen activator (t-PA). This drug helps to dissolve blood clots and restore blood flow. However, administration of t-PA is only suitable for about 5% of ischemic stroke patients due to the narrow therapeutic window [10, 11]. Intravenous tissue plasminogen activator (t-PA) thrombolytic therapy remains the only FDA-approved emergency drug treatment within 4.5 hours after acute ischemic stroke [12]. Unfortunately, the increased risk of intracerebral hemorrhage and the short treatment time window, limits the clinical use of T-PA [13, 14]. The pathogenesis of cerebral ischemia damage is complicated; This includes excitatory neurotoxicity, calcium overload, oxidative/nitrosative stress, and mitochondrial dysfunction as the principal mechanisms of cerebral ischemia injury [15, 16]. One of the main contributors to ischemic nerve cell damage is oxidative stress, which is affected by elevated calcium levels [17, 18]. Clutter of calcium homeostasis and its uncontrolled increment in nerve cells activate the generation of reactive oxygen species (ROS). ROS can also be constitutively generated by damaged mitochondria and by the activation of cell receptors that transmit signals from inflammatory mediators [19]. In patients with acute ischemic stroke (AIS), ROS are produced during the ischemic and reperfusion phases, which contributes to brain damage [20]. Intrinsic antioxidant potential cannot effectively neutralize reactive oxygen species (ROS). This leads to oxidative stress and disrupting the endogenous redox balance. Oxidative stress has a main role in the fundamental pathologic progression of cerebral damage in ischemic stroke [21, 22]. When oxidative stress occurs, ROS can oxidize lipids, proteins, and nucleic acids, leading to damage to the to the brain tissue and its cell structures [23, 24]. Furthermore, oxidative stress can trigger neuronal apoptosis, inflammation, and impairment of the blood-brain barrier, all of which exacerbate brain injury after an ischemic stroke [25, 26].

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland in the brain, and is primarily involved in regulating circadian rhythms of mammalians [27]. Researches have shown that melatonin is present in various parts of the body, including bile, cerebrospinal fluid, the anterior chamber of the eye, and ovarian follicular fluid. This suggests that melatonin may have functions beyond merely regulating the internal clock [28, 29]. Additionally, melatonin is recognized as an effective free radical scavenger and antioxidant [30]. The brain has considerable metabolic potential and interacts with a substantial amount of oxygen that enters the body. It is important to note that the brain contains high levels of iron, vitamin C, and unsaturated fatty acids [31]. However, due to the presence of the blood-brain barrier, the brain has limited access to free radical scavengers, making it vulnerable to oxidative stress damage. Acute ischemic stroke is considered as one of the external factors that can generate free radicals [32]. Recent studies have shown that exogenous melatonin can easily cross the blood-brain barrier with low toxicity and provide potential neuroprotective effects in a variety of central nervous system (CNSs) diseases [33]. Researches have indicated that melatonin plays a crucial role in enhancing cerebral blood flow following ischemic occurrences, reducing excitotoxicity, neuroinflammation, and ischemia-reperfusion injury [34-36]. Administration of melatonin during the acute phase can help reduce infarct size, inflammatory response, and oxidative damage [37]. The results of research studies have shown that antioxidant treatments can alleviate brain damage and improve this condition and its outcomes early on after an ischemic stroke occurs early after the occurrence of ischemic stroke can alleviate brain damage and also improve this condition and its outcomes [38-40]. Although numerous studies on the effects of melatonin on stroke have established its role as an antioxidant and anti-inflammatory agent, most of these have been performed on animal models and rodents. Several factors, including differences in the cardiovascular systems of humans and rodents, the presence of various subtypes of stroke among humans, and the diverse immune responses between patients due to differences in inflammatory status before the onset of stroke, generalization of these studies to humans without further investigations and additional clinical trials are unreasonable [41, 42].

Since few studies have assessed the effects of melatonin on improving the performance of ischemic stroke patients, we supposed that patients receiving melatonin early after ischemic stroke could be beneficial in improving their clinical status and performance, especially in patients not eligible for thrombolytic therapies [43]. Given the limited research in this area and the medical community's need for pharmacological treatments beyond thrombolytic drugs for managing ischemic stroke, further studies are essential. These investigations should focus on reevaluating the clinical

effects of melatonin at different doses and its effectiveness in enhancing patient outcomes.

Methods

Study design

This single-center, prospective, randomized, double-blind clinical trial with a placebo-controlled group was conducted on 70 patients with acute ischemic stroke who presented to the emergency department of Bu-Ali Hospital, affiliated with the Islamic Azad University, Tehran Medical Sciences Unit from December 2023 to November 2024. Preliminary screening was performed by a neurologist, confirming the diagnosis of acute ischemic stroke through neurological assessments, which was ultimately validated by magnetic resonance imaging (MRI) or computed tomography (CT) of the brain. The patients with acute ischemic stroke who were referred to the hospital and were not suitable candidates for thrombolytic therapy and mechanical thrombectomy were randomly assigned to the intervention and/or control group. Inclusion criteria were: a)18 to 90-year-old patients without regard to gender, b) occurrence of stroke within the past 24 hours, c) NIHSS score of at least 4, and at most 24, d) patients not eligible for thrombolytic therapy and thrombectomy. The exclusion criteria included: a) pregnancy or breastfeeding, b) acute or chronic intracranial hemorrhage or aneurism, c) any type of cognitive or behavioral disorder and substance abusers, d) concurrent inflammatory disease and/or malignancy, e) patients with transient ischemic attacks, f) sensitivity to melatonin, g) NIHSS score $\geq$ 25 or very severe stroke, h) presence of any nervous system disease that affects functional and neurological assessment, i) Severe cardiac or pulmonary disorders, hepatic (alanine transaminase levels more than three times the upper limit of normal), renal failure (serum creatinine 1.5 mg/dl or greater), j) The simultaneous presence of infectious diseases or a history of infection in the past month, k) unwillingness to participate in the study. All participants have written and filled out the informed consent before entering the study. The trial had been approved by the Iranian Registry of Clinical Trials (IRCT number: IRCT20231220060484N1).

Intervention

This clinical trial was designed with a consideration of a 10% dropout rate after applying the study exclusion criteria to 70 patients, based on the study by Mehrpooya et al. [43]. In the present study, the mean and standard deviation of NIHSS on day 90 were 7.78 and 2.14 in the intervention group and also 9.21 and 1.13 in the control group. Considering these results, the confidence interval of 95% and power of 90%, we estimated the required sample size to be 70 patients. Demographic data, past medical history, social history, and medication use before stroke were recorded for all participants at baseline. In this study, 35 patients received melatonin, while 35 patients were given a placebo. Usage of any medication that could interfere with clinical outcomes was restricted during the intervention. All of these patients received the initial management of acute ischemic stroke routinely based on AHA (American Heart Association) guidelines. [11, 44]. Moreover, rehabilitation therapies have been implemented to enhance the functional abilities of patients. [45]. Melatonin and a similar placebo were provided by Jalinous Pharmaceutical Company, Tehran, Iran. The melatonin treatment group received a 10 mg dose of melatonin at night, administered once within the first 24 hours after the onset of the stroke, and continued for five consecutive days to replicate the physiological function of melatonin in the body. The placebo group received a placebo treatment at night, which was administered once during the first 24 hours after the onset of a stroke and continued for five consecutive days (a gastric tube was used if necessary). At the end of the intervention period (day 5), both the melatonin and placebo treatments were discontinued. Patients were evaluated at the beginning of the study, five days after the intervention, and then again 30 days and 90 days later.

Effectiveness assessment

The effectiveness of treatment has been evaluated using the NIHSS and mRS criteria during scheduled examinations. The NIHSS is a criterion for assessing the severity of the stroke, the clinical effect of treatment, and neurological symptom variations over time. This scale consists of 11 components that are scored based on the physical and neurological conditions of patients [11, 46]. The final score ranges from 0 to 42, indicating the severity of the disease. In the present study, the neurological status of patients was evaluated initially and then during scheduled follow-up according to the NIHSS. Patients were classified into four clinical groups based on their scores: mild stroke (NIHSS score 0-8), moderate stroke (NIHSS score 9-15), severe stroke (NIHSS score 16-24), and very severe stroke (NIHSS score $\geq$ 25) [43]. The clinical status of patients was determined by the NIHSS criterion at the beginning of their enrollment in the study and then on days 5, 30±2, and 90±2 after the intervention and occurrence of the stroke. The degree of symptom improvement in patients was determined based on the analysis and interpretation of the data obtained. The AHA guidelines were used as a reference for calculating the NIHSS. The mRS is a scale that describes the degree of disability and is widely used in clinical studies to assess functional outcomes after a stroke [47]. This scale includes scores from 0 to 6, where a score of 0 indicates the absence of any disability, and a score of 6 indicates the death of the patient. In this study, to evaluate the degree of functional improvement and disability of patients, assessments were conducted at the beginning of

the study, 5 days post-intervention, 30 days post-intervention, and 90 days post-intervention based on the mRS criterion and relevant questions.

Adverse event assessment

A neurologist appraised the appearance of adverse effects during the intervention. This evaluation was carried out through patient reports and assessments of vital signs, laboratory data, and medical histories.

Randomization and blinding

The blocking process has two stages. In the first stage, samples were selected based on entry criteria through census, and in the second stage, the blocking method was used for randomization. The blocking process was carried out using random allocation software, and the block sizes were set to four. As a result, the total number of blocks was equal to 18. After obtaining informed consent, as predicted by the software, samples were assigned to one of the groups A or B. Additionally, to prevent the identification of groups A and B, each participant was assigned a random four-digit code obtained from the computer instead of labels A or B. The shape of the medications and placebos was completely identical. Thus, both the patients and the examining physician were unaware of the type of intervention, and the trial was conducted in a double-blind manner.

Statistical analysis

The data collected from the questionnaires were statistically analyzed by using SPSS software version 26. Results for quantitative data were reported as mean ± standard deviation, while qualitative data were reported as number (percentage). To compare the mean of quantitative variables (age, height, weight, body mass index, NIHSS score, and mRS) in patients with acute ischemic stroke receiving melatonin and placebo, an independent two-sample t-test was employed. Furthermore, to compare the distribution frequency of qualitative variables (gender, side effects, stroke origin, underlying diseases, risk factors, and medications used by patients) between the two study groups, the Chi-square test or Fisher's exact test was utilized.

To compare the slope of changes in the mean NIHSS score in patients with acute ischemic stroke receiving melatonin and placebo over the study period (before intervention, 5 days after intervention, 30 days after intervention, and 90 days after intervention), a two-way repeated measures ANOVA was used, and the interaction effect of group and time was assessed. The normality of the distribution of quantitative variables was assessed using the Shapiro-Wilk test, and the assumption of normality for the quantitative variables was confirmed ($P < 0.05$). Additionally, the normality of the distribution of quantitative variables was evaluated by calculating the skewness and kurtosis indices. Since the skewness and kurtosis values of the quantitative variables fell within the range of -2 to +2, the assumption of normality for the distribution of quantitative variables was confirmed. The equality of variances among the groups was assessed using Levene's test, and the homogeneity of the covariance matrices of the two groups was evaluated using Box's M test, both of which were confirmed ($P < 0.05$). The significance level in the tests was set at 0.05.

Results

This double-blind, randomized clinical trial was conducted from December 2023 to November 2024 on patients with acute ischemic stroke who were admitted to Bu-Ali Hospital. In this period, 114 patients with acute ischemic stroke presented to the treatment center, and among them, 70 patients met the inclusion criteria. They were randomly assigned to the melatonin or placebo group, in which each group contained 35 patients. During the study, one patient from each group died, but the remaining participants were evaluated until the end of the study (Figure 1).

Demographic characteristics

In the melatonin group, there were 17 women (48.6 %) and 18 men (51.4 %). There were 16 women (45.7 %) and 19 men (54.3 %) in the placebo group. The chi-square test indicated that the distribution of gender frequency between the two groups did not show a statistically significant difference ($P = 0.811$). The independent two-sample t-test demonstrated that the mean values of age, height, weight, and body mass index (BMI) of the stroke patients did not have statistically significant differences ($P > 0.05$). Therefore, the two groups are similar and homogeneous in terms of baseline characteristics (Table 1).

Baseline characteristic

In comparing the distribution of underlying diseases and the origin of stroke among patients receiving melatonin and placebo, Fisher's exact test and the chi-square test indicated that there is no statistically significant difference between both groups ($P > 0.05$). In comparing the frequency distribution of consumed medications, Fisher's exact test and the chi-squared test were utilized. The results indicated that the variable of ACEI and other drugs was significantly lower among patients receiving melatonin compared to those receiving a placebo ($P = 0.006$), while the distribution of other consumed medications between the two groups did not show a statistically significant difference ($P > 0.05$), (Table 2).

Clinical efficacy outcomes

After sequential evaluations of patients based on the NIHSS criterion, the independent two-sample t-test indicated that the average of NIHSS during the study

period (day one, day five, day thirty, and day ninety) did not show a statistically significant difference between melatonin and placebo recipients (P > 0.05). However, the average reduction of NIHSS from day five up to day thirty in patients receiving melatonin was significantly greater than that in patients receiving the placebo (P < 0.001). The independent two-sample t-test also revealed that the average reduction of NIHSS from day one up to day five and from day thirty up to day ninety did not show a statistically significant difference between both groups (P > 0.05), (Table 3). The two-way repeated measures ANOVA showed that the interaction effect between group and time is statistically significant (P < 0.001, F = 11.256). This means that the slope ofreduction in mean NIHSS over the study period differs significantly between patients receiving melatonin and those receiving the placebo. As shown in (Figure 2), during the period from day five up to day thirty, the slope of reduction in mean NIHSS in patients receiving melatonin is significantly greater than that in patients receiving the placebo.

In the comparison of mean mRS scores at different stages (baseline, day 5, 30, 90), the independent two-sample t-test indicated that there is no statistically significant difference between the melatonin and placebo groups (P > 0.05), (Table 4).

Safety and adverse events

In comparing the distribution of reported side effects, Fisher's exact test showed that the relative frequency of sleepiness disturbances in patients receiving melatonin was significantly higher than that in patients receiving the placebo (P= 0.022). However, the relative frequency of other reported side effects in patients with acute ischemic stroke did not show a statistically significant difference between the two groups (P > 0.05), (Table 5).

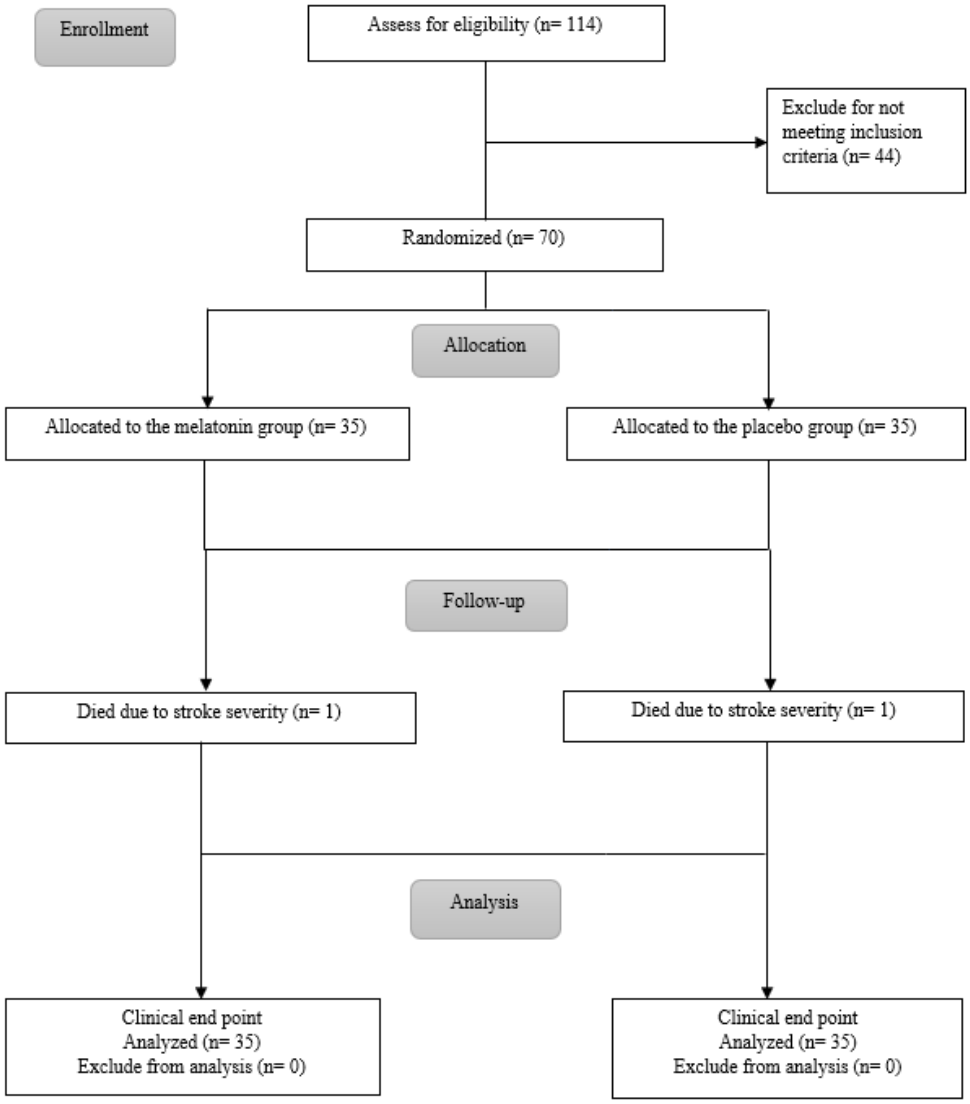

Figure 1- Flow diagram

Table 1- Comparison of demographic characteristics between both groups

Variable	Melatonin group (n= 35) Mean ± SD	Placebo group (n= 35) Mean ± SD	P value
Age (year)	68.09 ± 7.88	67.83 ± 8.27	0.894
Height (cm)	171.40 ± 7.85	171.94 ± 7.44	0.767
Weight (kg)	74.94 ± 10.80	74.77 ± 10.52	0.947
BMI (kg/m²)	25.55 ± 3.70	25.31 ± 3.28	0.773

Table 2- Comparison of baseline characteristics between both groups.

Variable	Melatonin group (n= 35) Number (%)	Placebo group (n= 35) Number (%)	P value
Diabetes	3 (8.6)	2 (5.7)	0.999
Diabetes and other risk factors	14 (40.0)	13 (37.1)	0.806
Hyperlipidemia	1 (2.9)	1 (2.9)	1.000
Hypertension	4 (11.4)	7 (20.0)	0.324
Hypertension and other risk factors	9 (25.7)	7 (20.0)	0.569
Smoking	2 (5.7)	1 (2.9)	0.999
Cardio-embolism	5 (14.3)	5 (14.3)	0.964
Large-artery atherosclerosis	21 (60.0)	19 (54.3)	
Small-vessel occlusion	4 (11.4)	5 (14.3)	
Other types	5 (14.3)	6 (17.1)	
ACEI[1]	5 (14.3)	1 (2.9)	0.198
ACEI and other drugs	1 (2.9)	9 (25.7)	0.006
ARB[2]	2 (5.7)	6 (17.1)	0.259
ARB and other drugs	5 (14.3)	3 (8.6)	0.710
Oral anti-diabetics and other drugs	10 (28.6)	7 (20.0)	0.403
Insulin, oral anti diabetics and other drugs	3 (8.6)	3 (8.6)	1.000
Statin and other drugs	2 (5.7)	0	0.493

1. Angiotensin converting enzyme inhibitors
2. Angiotensin II receptor blockers

Table 3- Comparison of mean NIHSS at different time measurement stages in both groups and Δ NIHSS.

Variable	Melatonin group (n= 35) Mean ± SD	Placebo group (n= 35) Mean ± SD	P value
NIHSS (day 1)	13.40 ± 4.39	13.09 ± 4.48	0.768
NIHSS (day 5)	12.54 ± 4.58	12.34 ± 4.72	0.858
ΔNIHSS (day 1-5)	0.86 ±0.73	0.74 ± 0.74	0.519
NIHSS (day 30)	9.03 ± 4.40	10.37 ± 5.00	0.241
ΔNIHSS (day 5-30)	3.56 ± 1.13	1.97 ± 1.86	<0.001
NIHSS (day 90)	7.26 ± 3.76	7.79 ± 3.67	0.559
ΔNIHSS (day30-90)	1.76 ± 0.86	2.15 ± 1.26	0.148

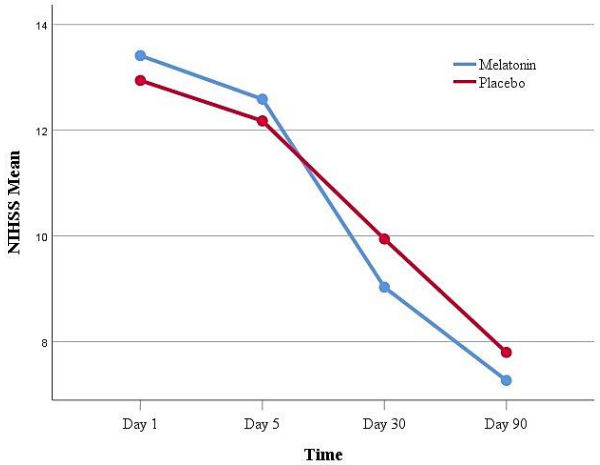

Figure 2- Comparison of the mean NIHSS at different time measurement stages in both groups.

Table 4- Comparison of mean NIHSS at different time measurement stages in both groups.

Variable	Melatonin group (n= 35) Mean ± SD	Placebo group (n= 35) Mean ± SD	P value
mRS day 1	4.34 ± 0.64	4.37 ± 0.77	0.866
mRS day 5	4.17 ± 0.66	4.31 ± 0.76	0.405
mRS day 30	2.80 ± 1.13	2.97 ± 0.92	0.490
mRS day 90	2.71 ± 1.13	2.91 ± 0.98	0.431

Table 5- Distribution of the frequency of reported side effects in both groups.

Variable	Melatonin group (n= 35) Number (%)	Placebo group (n= 35) Number (%)	P value
Dizziness	3 (8.6)	1 (2.9)	0.614
Dyspepsia	0	1 (2.9)	0.999
Headache	4 (11.4)	5 (14.3)	0.999
Insomnia	0	4 (11.4)	0.114
Nausea	2 (5.7)	2 (5.7)	1.000
Sleepiness	9 (25.7)	2 (5.7)	0.022
Nausea with Sleepiness	1 (2.9)	0	0.999

Discussion

Multiple risk factors, including hypertension, hyperlipidemia, diabetes, and smoking, are effective in the occurrence of stroke [48-50]. Most of the patients examined had at least one risk factor, with the majority of patients in both groups having diabetes and other risk factors (melatonin group: 40 %, placebo group: 37.1 %). Our data did not show any statistically significant differences between the two groups (Table 2).

Stroke is the sudden loss of blood circulation in a specific area of the brain, leading to the loss of neurological function related to that part of the brain [51-52]. The NIHSS is a standard scale for assessing the severity of stroke and evaluates the impact of stroke on patients' levels of consciousness, vision, speech, sensory, and motor function [53-54]. Mehrpooya et al. investigated the effects of melatonin on patients with acute ischemic stroke who were not suitable candidates for thrombolytic therapy. They concluded that administering 20 mg of melatonin within the first 24 hours after the onset of stroke has beneficial effects on the neurological and functional status of patients and observed a significant reduction in NIHSS scores one month and three months after the intervention [43]. This study demonstrated that the administration of melatonin has positive effects on the improvement of patients with acute ischemic stroke who are not suitable candidates for thrombolytic therapy. Considering the reduction in the NIHSS score on day 30, the improvement process of neurological disabilities in patients receiving melatonin is significant compared to the placebo group. However, our findings showed no statistically significant differences between the two groups three months after the intervention. The lack of a substantial reduction in the NIHSS score three months post-treatment may be related to the dosage of melatonin utilized or the duration of its administration. Administering melatonin at doses greater than 10 mg or for a duration longer than five days may yield better effects in improving patient outcomes (Table 3, Figure 1).

The occurrence of stroke leads to multiple damaging events, containing oxidative stress, neuroinflammation, mitochondrial dysfunction, breakdown of the blood-brain barrier, calcium overload, imbalance in excitatory and inhibitory neurotransmitters, and disruption of autophagy, which can ultimately result in neuronal damage and neurological deficits [43, 55-57].

Depending on the affected area of the brain, patients experience different clinical manifestations including functional disabilities, sleep disorders, depression, delirium, and so on [58-60]. In the study by Shreen and colleagues, melatonin at a dose of 3 mg was administered 24 to 48 hours after the occurrence of stroke, and patients were evaluated for six months based on the mRS, CASP (cognitive assessment for stroke patients), and CDS (communication disability scale) criteria. They found that melatonin, as a neuroprotective agent, led to improvements in the motor, speech, and cognitive status of the patients [61]. In this study, the degree of disability of patients was evaluated based on the mRS scale; however, no statistically significant difference was observed between the two groups receiving melatonin and the placebo (Table 4).

After a stroke, patients may face unpleasant outcomes that can affect their quality of life. In the examination of saliva samples of patients who experienced depression or sleep disturbances following stroke, it was observed that the melatonin secretion levels in these patients were altered compared to the control group [62-63]. Administering melatonin, a natural and safe substance, could significantly aid in improving their disorders [37, 64-65]. In the present study, based on the side effects reported by patients, it was found that no serious or dangerous adverse effects occurred. However, the side effect of sleepiness was reported more frequently among patients receiving melatonin. Our data showed a

statistically significant difference in the melatonin group compared to the placebo group (Table 5).

Multiple studies have investigated the effect of melatonin on ischemic models of rats and mice at varying doses, emphasizing its properties [66-68]. Melatonin reduced the damaged area of the brain and cerebral edema in ischemic models. Melatonin exerts its protective effects through various mechanisms, including lowering neuronal inflammation, glutamate excitotoxicity, oxidative stress, and alleviating mitochondrial dysfunction [69-72]. Several clinical trials have also revealed the potential impact of this substance at different doses on improving outcomes following stroke. Due to its inherent characteristics, melatonin may be effective alongside routine stroke treatments [34, 37, 43, 61, 73].

The patients participating in this study were selected based on defined inclusion criteria, and it was conducted in a treatment center with a limited number of patients, which is among the limitations of our study.

Conclusion

Melatonin's anti-inflammatory and antioxidant properties make it a neuroprotective agent in post-stroke treatments. It reduces the NIHSS score and improves sensory and motor disabilities in patients with acute ischemic stroke.

Ethical Approval

IR.IAU.PS.REC.1402.522

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