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NOVEL CONCEPT

Chronic Supplementation of Melatonin restores Impaired Circadian Rhythm in Patients with Coronary Artery Disease

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ABSTRACT

Blood pressure (BP) has a characteristic and reproducible circadian pattern with high values during the day and low values at night. Previous studies have shown that in patients with coronary artery disease (CAD), the nocturnal dip of BP is absent or blunted, which may be correlated to the reduced melatonin levels or altered melatonin–cortisol interplay. Our objective was to assess the effect of bedtime melatonin administration on circadian pattern of BP and heart rate (HR) in CAD patients. One hundred CAD patients were recruited for the study. General health records were individually maintained. Each study participant was given a 5 mg pure melatonin supplement each night at bedtime for a period of 1 year. A 24 hour/7 day ambulatory blood pressure monitoring (ABPM) using ambulatory blood pressure monitor and serum melatonin level estimations were done initially, after 6 months, and after 1 year of melatonin supplementation. The rhythmic parameters of systolic BP (SBP) and diastolic BP (DBP), HR, viz. midline-estimating statistic of rhythm (MESOR), double amplitude, acrophase, 3 hour fractionated hyperbaric index (HBI) were significantly reduced and serum melatonin concentration significantly increased after 6 and 12 months of exogenous melatonin supplementation. Circadian hyperamplitude tension (CHAT) incidence decreased as melatonin treatment progressed. The number of subject diagnosed with CHAT was as follows: 37/100 at the beginning, 17/100 after 6 months, and 6/100 after 12 months. These data suggest that 5 mg/day melatonin treatment improved and restored the circadian pattern of BP in CAD subjects.

Keywords: Ambulatory blood pressure monitoring, Circadian hyperamplitude tension, Coronary artery disease, Hyperbaric index, Melatonin.

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INTRODUCTION

Most physiological parameters exhibit circadian rhythms reflecting an innate temporal program provided by biological clocks. Chronobiological studies include but are not limited to comparative anatomy, physiology, genetics, molecular biology, and behavior of organisms within biological rhythms mechanics. Systemic BP has a characteristic and reproducible daily pattern, as regulated by the circadian timing system.[1,2] In most people, BP drops by 10 to 20% during night are called dippers; those in whom such reductions are not present appear to be at increased risk for cardiovascular events and these peoples are called nondippers. In addition, it was reported that in people whose 24-hour BP exceeded 135/85 mm Hg, were nearly twice as likely to have a cardiovascular event as those with 24-hour mean BPs <135/85 mm Hg, irrespective of the level of the office BP.[3] The regular cycle of BP is important especially because an inverted daily pattern with higher BP values during the night and lower during the day is associated with an increased target organ damage (in cardiac, cerebral, vascular, and renal tissues) and worsened cardiovascular outcome. Patients with CAD, a major complication of chronic hypertension, show a blunted day–night rhythm in vasodilatation and suppressed or attenuated night-time melatonin levels,[4] and thus the normal dipping pattern of BP is absent or disturbed.[4,5,8,9] Melatonin is a circadian clock-regulated hormone produced largely from the pineal gland. Mounting evidence reveals that the rhythmicity of melatonin has a crucial role in a variety of cardiovascular pathophysiological processes including anti-inflammatory, antioxidant, and antihypertensive and, possibly, antilipidemic functions.[6,7] Normally, melatonin levels are low during the day and high at night.[6] A possible link between melatonin and circulation is suggested by the following: (i) The rhythms in melatonin and cardiovascular activity have inverse phase relationship. The nocturnal rise in melatonin coincides with the declined cardiovascular activity.[8,9] (ii) In experimental animals, melatonin is shown to prevent ischemia and/or reperfusion-induced cardiac arrhythmias, influence BP control,[10,11] regulate blood flow to the brain,[12] and modify peripheral artery responsiveness to norepinephrerine.[13] A melatonin supplement at night increases endogenous melatonin levels and hence, helps to protect from cardiovascular diseases.[14] Zonobani A et al (1978)

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further suggested that melatonin by its action on the suprachiasmatic nucleus (SCN), the circadian clock influences the autonomic output to the cardiovascular system[15] and in turn improves the circadianly regulated autonomic regulation of BP in hypertensive patients. Hence, it can be reasoned that low melatonin levels are linked with the impairment of the cardiovascular functioning. In humans, melatonin production not only diminishes with age, but also lowers in many age-related diseases, including cardiovascular disease.[16,17] Coronary artery disease patients show a markedly decreased nighttime melatonin synthesis,[17] which may be assumed reason for absence of nocturnal dip in BP and high chances of myocardial infarction.[5] In addition, light/dark variations in the production of endogenous inflammatory markers in patients with CAD to some extent may be related to day/night fluctuations in circulating melatonin levels.[6] Melatonin secreted from pineal gland and controlled by SCN plays a vital role in maintaining normal circadian pattern[18] and also in autonomic regulation of cardiovascular system.[19] There is a growing body of evidence suggesting that a blunted decrease in nighttime BP is associated with a greater risk of target organ damage. It has been suggested that impairment of the sympathetic nervous system may contribute to the attenuation of the nocturnal BP dip.[20] However, the mechanisms underlying this abnormal nighttime BP dipping pattern are not fully understood.[16] There is some evidence suggesting that a nondipping BP pattern may be related to advanced age,[21] sodium sensitivity,[22] postmenopausal status,[20] sleep apnea, and poor sleep quality.[21] Some studies state that melatonin supplementation at certain times during the night may function to increase endogenous melatonin level, potentially protecting against the melatonin downregulation associated with cardiovascular diseases.[44,47] Because melatonin via SCN influences the autonomic output to the cardiovascular system,[15] restoration of proper functioning of the SCN in patients with hypertension could improve the autonomic regulation of BP. In this study, we used a 7 day timed analysis of the records of BP through ABPM to diagnose day-to-day variability,[23] which is termed as CHAT, a condition in which excessive circadian BP amplitude precedes chronic established hypertension.[24] We therefore, investigated in a 1 year study the effect of bedtime melatonin supplementation on the circadian pattern of BP in patients with CAD and established the clinical implications of it on 24 hours/7 days rhythmic pattern of BP in CAD patients.

MATERIALS AND METHODS

Registration of Volunteer Patients of CAD

One hundred patients of CAD diagnosed by invasive or noninvasive techniques at King George Medical University, Lucknow, India, were registered for the present study. The protocol of this study was approved by the Committee of medical Ethics, Research cell CSMMU (3118/R.Cell-08), Lucknow, in compliance with the declaration of Helsinki principles of medical ethics. All the subjects were explained about the purpose and protocols of the study before obtaining written informed consent from each of them in English and Hindi languages. Statistical significance between initial, 6-month and 1-year experimental values was compared for analysis. No separate control group was recruited to avoid any medical/pathophysiological changes during statistical comparisons.

Treatment

All study participants took 5 mg melatonin as supplement, orally before sleep for a period of 1 year. The supplement was pure melatonin procured from Marc Laboratories, Himachal Pradesh, India. All study participants were allowed to continue their ongoing treatment.

Monitoring of Health Records

The health records of each subject were maintained during the entire period of treatment with general observations, like the presence or absence of headache, insomnia, hyperactivity, irritability, nausea, sleeping limbs, dizziness, constipation, shaky hands, stomach cramp, drowsiness, sweating, hunger, weakness, and sore eyes. The participants were instructed to report immediately in case they developed symptom(s) of any of these diseases, and advised to discontinue the oral intake of melatonin.

Ambulatory Blood Pressure Monitoring

A 24 hour/7 day ABPM of the subjects was done with an automated ABPM device, A&D TM-2430 (A&D Company, Japan). The ABPM of each subject was done three times before and after 6 and 12 months of the oral melatonin supplement. Participants were told to carry out all their routine works during recording periods. The machine was programmed to record BP and HR taken every 30 minutes during the day and after 1 hour interval during the night. Measurements from the ABPM device were transferred and stored in the computer for further analysis. For each individual, the data were summarized in a sphygmochron (a computer comparison of patients' profile with the specified reference limit). The results were analyzed using Halberg Cosinor analysis. Each BP and HR profile was analyzed by a sphygmochron, utilizing both a parametric and nonparametric approach. Ambulatory blood pressure monitoring records were sent to Halberg Chronobiology Center, University of Minnesota, and Minneapolis, USA, for further interpretation. The following estimates were obtained: (i) MESOR, a time structure

Table 1: Clinical characteristics and observation of CAD subjects enrolled for the study

Clinical characteristics	*Males*	*Females*	*All subjects*
No. of subjects	69	31	100
Age (years; mean ± SE)	62.2 ± 13.4	58 ± 10.44	65.44 ± 13.54
Height (cm; mean ± SE)	173.73 ± 4.52	158.49 ± 4.38	170.25 ± 9.42
Weight (kg; mean ± SE)	62.32 ± 8.26	59.68 ± 7.22	60.26 ± 8.35
BMI (kg/m^2; mean ± SE)	22.37 ± 2.50	20.17 ± 1.89	25.56 ± 3.53
Casual SBP (mm Hg; mean ± SE)	142.21 ± 12.32	139.35 ± 6.23	140 ± 11.20
Casual DBP (mm Hg; mean ± SE)	88.26 ± 7.1	85.63 ± 2.33	87.63 ± 3.66
Casual heart beats (bpm; mean ± SE)	85 ± 11	92 ± 9	87 ± 11

Values represent the mean ± SE of 100 subjects; SE: Standard error

or chronome-adjusted mean;[25] (ii) double amplitude or predictable change, which is the total change within a day or the circadian amplitude of reproducible variability within a day,[9] (iii) acrophase, which is a measure of timing of overall high values recurring in each cycle; (iv) CHAT. The average 7-day values of the MESOR, double amplitude, acrophase, and 3-hour fractionated HBI for SBP, DBP, and HR were determined for each subject at all the three recordings, i.e., initial, after 6 and 12 months. The CHAT was diagnosed in subjects who were having larger-than-usual change in BP, and overswinging BP pattern in the double amplitude which precedes an overall elevation in BP and is an indication or predictor of a great risk of essential hypertension and CAD.

Serum Melatonin Estimation

Blood samples of CAD subjects were collected between 8:00 and 10:00 AM for the estimation of serum melatonin levels by enzyme-linked immunosorbent assay kit procured from IBL International, Germany.

Statistics

Statistical significance between initial, 6 months and 1 year experimental values of ABPM were calculated by Halberg Cosinor analysis and Student's t-test,[9,25-27] whereas melatonin hormone analysis was calculated by Student's t-test only.

RESULTS

Clinical Observations

Twenty of the 120 patients dropped out of the study. There were 69 males and 31 female subjects (mean age 60.44 years). Mean SBP/DBP of the subjects recorded by mercury sphygmomanometer was 140 ± 11.20/87.63 ± 3.66. Mean HR was 87.63 ± 3.66 bpm (Table 1).

Ambulatory Blood Pressure Monitoring

Midline-estimating Statistic of Rhythm

There was significant difference between the baseline MESOR of SBP, DBP, and HR after 6 months and 1 year of the supplementation of melatonin in CAD subjects. In CAD patients, baseline SBP/DBP was 142.07 ± 1.18/92.40 ± 2.77, which was significantly reduced to 133.03 ± 4.36 after 1 year of melatonin supplementation. Melatonin significantly reduced SBP. A 3% decrease was noted after 6 months and 6.3% decrease was noted after 1 year of melatonin supplementation. The decrease of 3.3% after 6 months and 8.2% after 1 year was observed in DBP. There was a 5.58% decrease after 6 months and 9.63% decrease after 1 year in MESOR HR, which was highly significant (Table 2 and Graph 1).

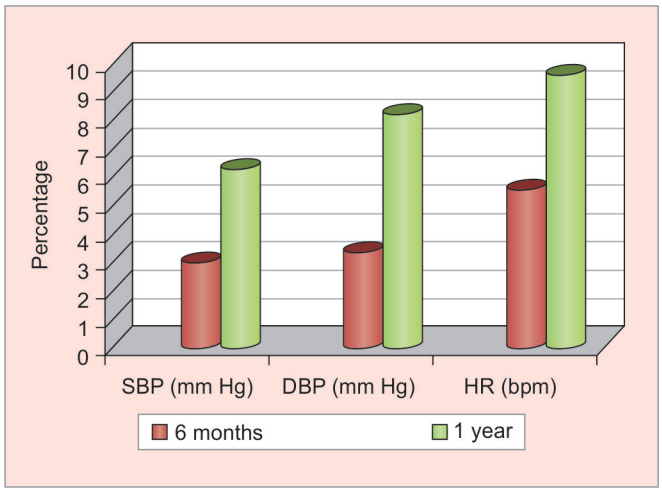
Double Amplitude (Predictable Change)

The circadian amplitude of reproducible variability also decreased significantly after 6 months and 1 year of the supplementation of melatonin in CAD subjects. There

Table 2: MESOR of 24 hours/7 days ABPM in CAD subjects initially and after 6 months and 1 year of melatonin supplementation

	Initial	*6 months*	*1 year*
SBP (mm Hg)	142.07 ± 1.18	136.70 ± 2.86* ($p<0.05$)	133.03 ± 4.36*Δ ($p<0.01$)
DBP (mm Hg)	92.40 ± 2.77	88.86 ± 6.84* ($p<0.05$)	84.82 ± 7.63*Δ ($p<0.01$)
HR (bpm)	83.592 ± 0.94	78.92 ± 0.612* ($p<0.05$)	75.05 ± 0.358*Δ ($p<0.01$)

*Significant at the level of $p<0.05$; Δ Significant at the level of $p<0.01$



Graph 1: Percent decrease in MESOR of SBP, DBP, and HR after 6 months and 1 year of melatonin supplementation in CAD subjects

Table 3: Double amplitude (predictable change) of 24 hours/7 days ABPM in CAD subjects initially and after 6 months and 1 year of melatonin supplementation

	Initial	*6 months*	*12 months*
SBP (mm Hg)	28.04 ± 0.98	23.14 ± 0.91*Δ ($p<0.01$)	20.91 ± 0.79*Δ ($p<0.01$)
DBP (mm Hg)	19.68 ± 0.84	17.94 ± 0.61* ($p<0.05$)	13.04 ± 0.65*Δ ($p<0.01$)
HR (bpm)	16.66 ± 0.697	12.95 ± 0.98*Δ ($p<0.01$)	10.57 ± 0.46*Δ ($p<0.05$)

*Significant at the level of $p<0.05$; Δ Significant at the level of $p<0.01$

Table 4: Acrophase of 24 hours/7 days ABPM in CAD subjects initially and after 6 and 12 months of melatonin supplementation

	Initial	*6 months*	*12 months*
SBP, mm Hg (hh:mm:ss)	14:51 ± 0.233	15:54 ± 0.141	16:33 ± 1.14
DBP, mm Hg (hh:mm:ss)	15:20 ± 0.92	14:50 ± 0.206	13:41 ± 0.228
HR, bpm (hh:mm:ss)	15:45 ± 0.43	14:20 ± 0.215	15:24 ± 0.207

Values represent the mean ± standard error of 100 subjects at each time interval

was 10% decrease in SBP, 20% in DBP, and 10% decrease in HR after supplementation (Table 3 and Graph 2).

Circadian Hyperamplitude Tension

Circadian hyperamplitude tension (CHAT) was diagonsed in 37/100 subjects. After 6 months and 1 year of melatonin supplementation, CHAT was demonstrated in 17 and 6 cases respectively ($p = 0.05$; Graph 3).

Acrophase

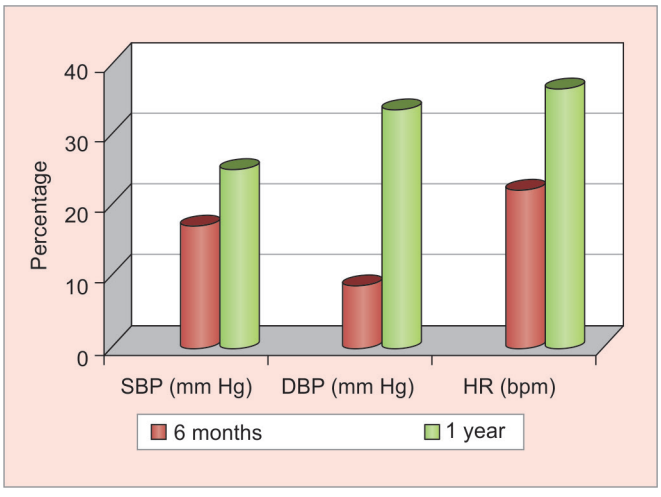
There were no statistically significant changes in acrophase of initial, 6, and 12 months ABPM of CAD subjects. However, the circadian pattern of timing of overall high values recorded at baseline (SBP/DBP 14:51 ± 0.23/15:20 ± 0.92) recurring in each cycle shifted toward normal (SBP/DBP 16:33 ± 1.14/13:41 ± 0.22) after 12 months of supplementation of melatonin. There was no significance difference between the initial and 12 months supplementation values of acrophase of HR (Table 4).

Serum Melatonin

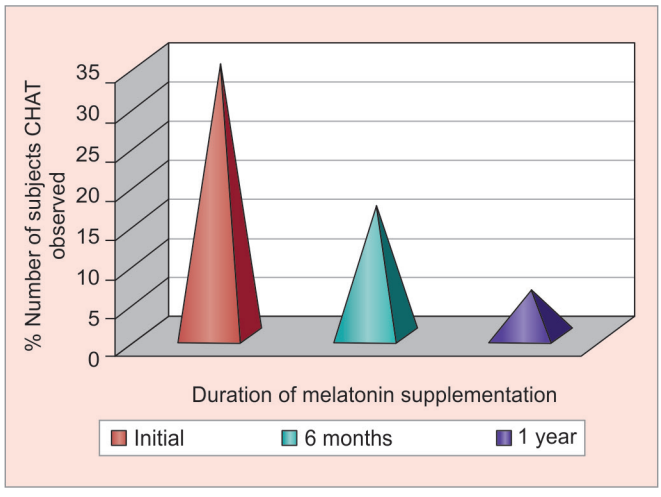
Significant increase in serum melatonin levels was noted after 6 and 12 months of melatonin supplementation. Graph 4A shows the total increase in serum melatonin level and Graph 4B shows the effect of serum melatonin levels on the SBP, DBP, and HR.

DISCUSSION

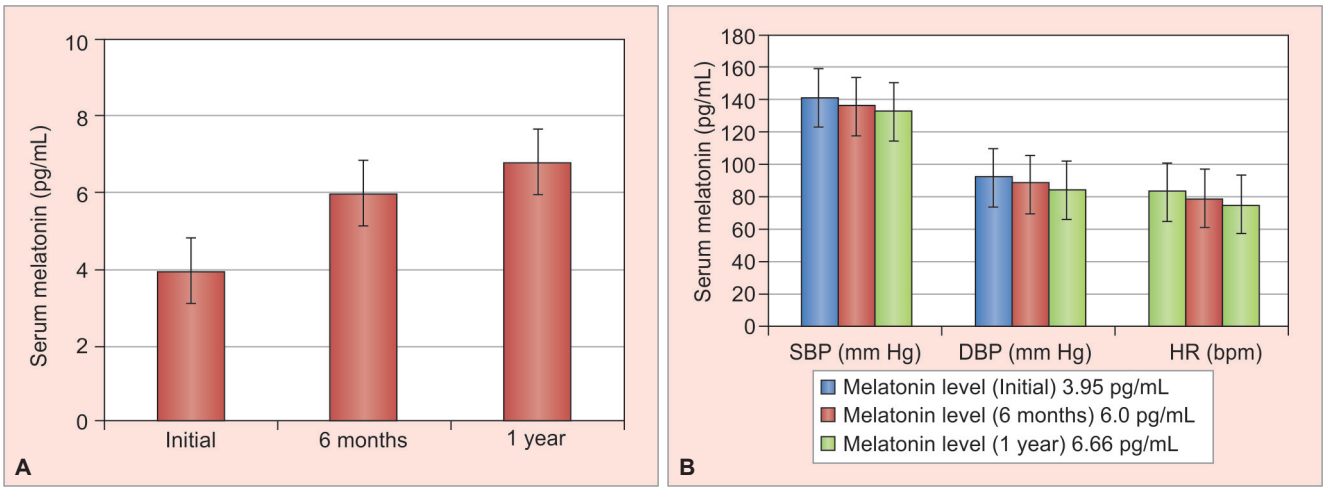
Baseline observation of our study shows that CAD patients had disturbed circadian pattern of 24 hours/7 days ambulatory BP, which were restored or improved after they took melatonin as supplement. One year of melatonin as supplementation significantly reduced ABP and increased serum melatonin level (Table 2 and Graph 1). A reduction of 3 mm Hg of systolic MESOR was observed after 6 months, which was further decreased to about 9 mm Hg after 12 months. Similarly, after 12 months, DBP was reduced to 4 to 7 mm Hg and MESOR HR was reduced to 5 to 8 bpm. This is consistent with the previous reports that melatonin repeatedly given orally or intranasally can reduce BP with essential hypertension.[28,29] However, none of the subjects had complained of headache, palpitation, dyspnea, nausea, tremors, dizziness, sore eyes. There was initially high BP amplitude in 35% of our CAD subjects (Graph 3), which is the predictor of the CHAT and vascular disease risk associated with it.[25,30] We found



Graph 2: Percent decrease in double amplitude (predictable change) of SBP, DBP, and HR after 6 months and 1 year of melatonin supplementation in CAD subjects



Graph 3: Percentage of number of subjects in whom CHAT was diagnosed during ABPM (n = 100)



Graphs 4A and B: (A) Total increase in serum melatonin level; and (B) effect of serum melatonin levels on the SBP, DBP, and HR

that after 6 and 12 months of the melatonin supplement, CHAT was reduced to 17 and 6% respectively, and non-dipping pattern shifted toward dipping. Recent findings of Rechciński et al[31] also suggest nocturnal decline in BP of CAD patients, but there was also a nonoptimal increase in the daytime BP. We have found that melatonin given as supplement causes nocturnal decline in ambulatory dipping pattern without a nonoptimal increase in the daytime BP. There was no significant difference between the acrophases (time of excess) of BP and HR before and after the melatonin supplement. Circadian rhythm of acrophases (high values) lie at an interval of 12 to 20 hours, and batyphases (lowest values) at an interval of 2 to 6 hours.[32] Similar pattern was observed in the acrophase (Table 4) of subjects after 12 months of oral melatonin, suggesting the synchronizing role of melatonin for daily BP rhythms. Our results indicated a significant increase in serum melatonin concentrations of CAD subjects after 6 and 12 months of melatonin supplementation. However, initial observation in same subjects indicated low levels of serum melatonin. Low levels of melatonin in CAD subjects have also been already reported.[5,33] The rise in serum levels of endogenous melatonin of CAD subjects after melatonin supplement may be due to the improvisation of SCN autonomic activity possessed by exogenous melatonin supplementation. Previously Laakso et al[34] reported increase in endogenous melatonin level after administration of prolonged exogenous melatonin in young and old people. Melatonin may provide feedback via high-affinity melatonin receptors in the SCN,[35,36] thus influencing the rhythm of its own production and other circadian rhythms too.[14,37] The mechanism of protective effect of melatonin has been discussed by many authors. Being a lipophilic molecule, melatonin can effect intracellular MT1 and/MT2 receptors[38] found in the cardiovascular system.[39] The constrictive effect of melatonin can be explained by receptor-mediated decrease in the cyclic adenosine monophosphate levels[40,41] and phosphatidylinositol-4, 5-bisphosphate hydrolysis.[38] During initial observation of our study, hypertension was observed in maximum number of CAD subjects; this may be associated with disturbed neurotransmission in SCN,[42] which is the regulatory center for melatonin secretion[43] and autonomic tone.[44,45] Our findings implicate that melatonin influences central regulatory mechanisms involved in the BP control, as indicated by reported restored baroreflex responses,[45] decreased sympathetic output,[46] and associated decreased HR or cardiac output with BP fall after melatonin administration[14,47] in other studies. The mechanisms participating in central effect of melatonin are yet not completely known; however, several pathways are suggested by many authors. It could be hypothesized that the modulation of SCN activity by melatonin[36,38] alters sympathetic tone and represents a protective mechanism against excessive sympathetic excitation. Thus, our studies suggest a protective role of melatonin in synchronization of impaired circadian pattern of BP in subjects with CAD. Normal ABPM is itself a strong predictor of enormous variability of BP and HR. Extent of hour-to-hour and day-to-day variability, 24 hours/7 days monitoring has emerged as an important tool of studying variability in BP, which helps in prognosis of hypertension and white coat hypertension. We recognize that melatonin is of special interest, being an endogenous molecule that can be used in humans, and which is also safe. In the future, more experiments are required to study the effect of melatonin on cardiovascular system and elucidate the potential interactions between melatonin and the different classes of antihypertensive medicine in BP regulation. Since cardiovascular regulation is a complex mechanism which may not be solely regulated by melatonin, we will like to conclude that melatonin offers a protective role in subjects with dissynchronous circadian pattern of cardiac functions

and could minimize the risk of various clinical events associated with BP rhythms.

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