PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

www.pacjmedsci1625.com. Email: managingeditorpjms1625@gmail.com.

EFFECT OF LIGHT RESTRICTION AND NIGHTLIGHT EXPOSURE ON PLASMA MELATONIN LEVELS, OXIDATIVE STRESS, AND INFLAMMATORY BIOMARKERS IN FEMALE WISTAR RATS

(Running title: *Effect of Nightlight and Constant Darkness Exposures on Plasma Melatonin Levels, Oxidative Stress and Inflammatory Biomarkers in Female Wistar Rats*)

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ABSTRACT

The objective of this study was to investigate the effect of nightlight and constant darkness exposures on plasma melatonin levels, oxidative stress, and inflammation in female Wistar rats. Fifteen female Wistar rats (120 – 150 g) were randomly divided into three groups (5 rats per group), namely Control, Nightlight, and Darkness. The Control group, represented as G-1, was exposed to 12:12 h light/dark cycle. The two experimental groups were Nightlight group (G-2) and Darkness group (G-3). The Nightlight group (G-2) was exposed to 24 h light, and the Darkness group (G-3) was exposed to 24 h darkness. The experiment was carried out for 6 weeks. The animals were sacrificed, and the oxidative and inflammatory parameters were evaluated. Plasma melatonin levels in the rats decreased significantly in G-2 compared to G-1, while G-3 showed higher melatonin levels compared to G-1. The rats in G-2 had increased plasma malondialdehyde (MDA) and decreased superoxide dismutase (SOD) levels compared to those in G-1. The rats in G-3 had reduced MDA levels and increased catalase (CAT) levels compared to G-1. Proinflammatory marker, interleukin-6 (IL-6) decreased in G-2 while G-3 had higher IL-6 level compared to G-1. The result suggests that extreme light exposures alter the plasma melatonin secretion, resulting in oxidative stress and inflammation.

Keywords: Nightlight Exposure, Darkness Exposure, Melatonin, Oxidative Stress, Inflammation.

Manuscript Submitted: May 2024; Accepted: July 2024

INTRODUCTION

The natural light-dark cycle of the Earth, which is governed by its rotation, serves to synchronize the circadian rhythms that control behaviors and physiological processes in most organisms [1]. Entrainment to this light-dark cycle ensures coordination between the central hypothalamic circadian clock and the peripheral circadian oscillators. Currently, the extensive use of artificial lighting has resulted in the phenomenon of "light pollution," which disrupts the natural circadian rhythms in a significant proportion of the global population [2].

The detrimental consequences of excessive exposure to artificial light during the night are increasingly apparent and linked to various health complications [3,4]. Moreover, the brightness emitted by urban nightscapes and electronic devices has resulted in a modern-day environmental issue referred to as light pollution [4,5]. Conversely, limited exposure to light, such as prolonged periods of darkness, can also disturb circadian rhythms, leading to irregular oscillations in circadian parameters [6,7]. Environmental factors, such as dietary patterns and altered light/dark cycle, contribute to a higher prevalence of obesity, metabolic syndrome, and chronic low-grade inflammation. It is noteworthy to mention that these factors also have a significant impact on the reproductive endocrine functions in women who are in the age range of childbearing [8]. Although constant darkness has been proposed as a circadian metabolic signal in mammals [9], there is however insufficient comprehensive data on its effects on endocrine function during prolonged periods of constant darkness.

Circadian timing is influenced by both an individual's circadian rhythm and the lighting environment in which they are situated [10]. Light primarily affects the circadian clock through the activation of the eye's intrinsically photosensitive retinal ganglion cells (ipRGCs). These ipRGCs serve as photoreceptors expressing melanopsin (a photopigment), which exhibit the highest sensitivity to light at approximately 480 nm [11]. Upon stimulation, these ipRGCs transmit signals via the retino-hypothalamic tract to the suprachiasmatic nucleus (SCN), the central circadian clock. As a result, the SCN regulates the pineal gland secretion of melatonin, a hormone that facilitates sleep [12]. Studies indicate that this mechanism becomes apparent early in the developmental stages of mammals [13,14]. The adult response to light in terms of circadian rhythms is dependent on light intensity, and even low levels of evening light exposure can suppress melatonin production [15,16]. Artificial night-time lighting significantly disrupts the natural secretion of melatonin, a crucial component of the circadian cycle that is typically produced during darkness [8]. Constant exposure to light, as demonstrated

in female rats' study, has been shown to have a substantial impact on the development of mammary tissue [17].

Disruption of melatonin production due to environmental factors like night work is considered a potential risk factor for diseases, such as, malignant tumors in the breast [3]. Considering both experimental and epidemiological findings related to breast cancer, the International Agency for Research on Cancer (IARC) has categorized nightwork involving shifts linking circadian disruption or chrono-disruption as a likely human carcinogen [3].

Melatonin is a versatile molecule found across various organisms, ranging from plants, lower animals to humans. The synthesis primarily occurs during dark periods in the pineal gland and is regulated by the light/dark cycle through the SCN [18]. This indolamine, which is derived from L-tryptophan plays several important roles in regulating circadian periods. Beyond its action in timekeeping, melatonin is known for its apparent antiapoptotic signaling function and acts as a potent antioxidant [18]. These cytoprotective properties hold potential implications for treating neurological disorders. In addition to these functions, melatonin also has anti-cancer and immune-boosting effects [3,19].

Melatonin is present in high concentrations in various tissues where it can, through paracrine signaling, potentially influence other physiological functions [20]. Moreover, melatonin exhibits anti-inflammatory effects during acute inflammation [21,22] and plays a role in regulating the hypothalamic-pituitary-gonadal axis function [23]. Additionally, melatonin acts, sometimes, by binding to its specific receptors, melatonin receptor 1 (MT1) and MT2) on target organs, both coupled to Gi-proteins (Giαβγ) [24].

This present study investigated the impact of extreme light exposures on melatonin actions on oxidative stress, inflammatory markers, and reproductive hormones in female Wistar rats.

METHODOLOGY

Experimental Animals and Grouping:

All aspects of animal handling and experimental procedures in this study adhered to the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals [25]. Ethical clearance for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC919/21).

Fifteen female Wistar rats, with body weights ranging from 120 to 150 grams, were acquired from the animal housing facility at the Department of Physiology, Babcock University, Ilishan-Remo, Nigeria. These rats were provided with ad libitum access to standard rat chow and tap water. The animals were then randomly distributed into three groups, with each group containing five rats per group.

Experimental Design:

After 2 weeks of acclimatization, the three groups were Control group (G-1), Nightlight group (G-2) and Darkness group (G-3).

G-1 was exposed to a 12:12 h light/dark cycle (lights on at 6:00 and off at 18:00).

The cage of G-2 was altered, and a 230-Volt LED Lamp with an E27 base (10 watt, 806 Lumens, 3000 Kelvins) was installed for 24 hours artificial light. The cage of G-3 was covered with a dark film for 24 hours constant darkness. This experiment was adapted from Farhadi et al. and Faborode et al and extended for a period of 6 weeks [26,27].

Sample Preparation:

Following 6 weeks of the experiment, the animals underwent a 12-hour overnight fast. Blood samples were then retro-orbitally collected into heparinized tubes and centrifuged at room temperature for 15 minutes at 3000 rpm. The resulting plasma was carefully separated and stored in a frozen state until required for biochemical analysis. Subsequently, the animals were sacrificed via cervical dislocation.

Biochemical assays

Oxidative stress markers:

Plasma malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels were assessed according to the manufacturer's procedure [27].

Inflammatory Biomarkers:

Quantitative standard sandwich ELISA technique was employed to determine the levels of tumor necrosis factor-alpha (TNF-α) [27] and interleukin-6 (IL-6) in the plasma. Monoclonal antibodies specific for these parameters were utilized, and rat kits sourced from Elabscience Biotechnology 14780 Memorial Drive, Suite 216, Houston, Texas, were employed for the analysis.

Plasma Hormone Concentration:

Plasma melatonin, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, and estrogen levels were quantified using Rat ELISA kits from Monobind Inc. Lake Forest, CA 92630, USA. The assay procedures provided by the manufacturers were followed for analysis.

Statistical analysis**:**

Statistical group analysis was conducted using GraphPad Prism 9.5.1. All data were presented as means ± SEM.

To compare variable means among groups, a one-way ANOVA was employed, followed by post hoc analysis using Bonferroni's test. Statistically significant differences were considered when p-values were less than 0.05.

RESULTS

Effect of Nightlight and Darkness exposures on plasma melatonin levels in Female Wistar rats:

There was significant decrease ($p<0.05$) in plasma melatonin levels in the nightlight group (G-2) compared to the control group (G-1). However, there was a significant increase ($p<0.05$) in the darkness group (G-3) compared with the nightlight group (G-2) (Fig 1).

Effect of Nightlight and Darkness exposures on Malondialdehyde in Female Wistar rats:
There was significant increase ($p <0.05$) in plasma MDA level in the nightlight group compared with the control group. However, there was significant decrease ($p <0.05$) in plasma MDA level in darkness group compared with the Nightlight and control groups (Fig 2).

Effect of Nightlight and Darkness exposures on Antioxidant markers in Female Wistar rats:
There was no significant decrease ($p <0.05$) in plasma CAT in nightlight group, however, darkness group was significantly increased ($p <0.05$) when compared with nightlight and control groups. There was significant decrease ($p <0.05$) in SOD level in the Nightlight group compared to the control group. Interestingly, darkness group also had significant decrease ($p <0.05$) when compared with control group (Fig. 3).

Effect of Nightlight and Darkness exposures on Inflammatory makers in Female Wistar rats:
There were no significant changes in plasma TNF-α levels across all groups. There was a significant decrease ($p <0.05$) in IL-6 level in the nightlight group compared with the control group. However, plasma IL-6 levels in the darkness group had a significant increase ($p <0.05$) compared with the nightlight and control groups (Fig. 4).

Effect of Nightlight and Darkness exposures on Reproductive Hormones in Female Wistar rats:
There was no significant difference in plasma FSH, LH and Estrogen in the Nightlight group across all groups. However, there was significant decrease ($p <0.05$) in progesterone concentrations in nightlight group against control and decrease against darkness group (Table 1).

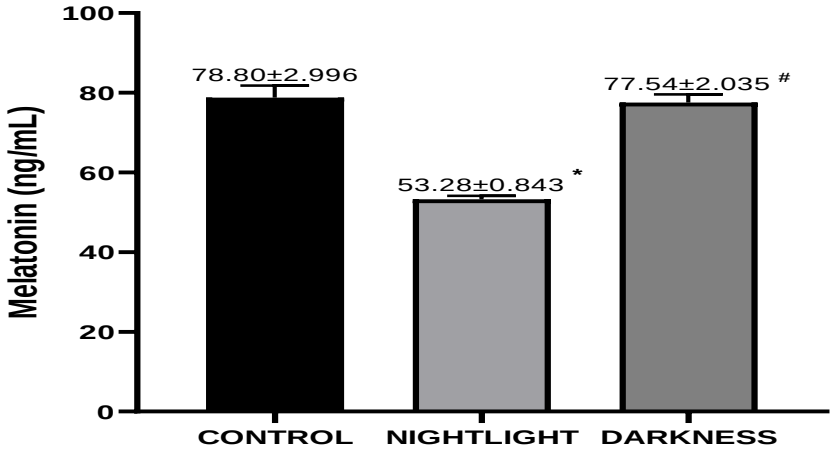

Fig 1: Effect of Nightlight and Darkness exposures on plasma melatonin levels in Female Wistar rats. Data are expressed as mean ± S.E.M. n=5. (**p*<0.05 VS. CONTROL; #*p*<0.05 VS. NIGHTLIGHT)

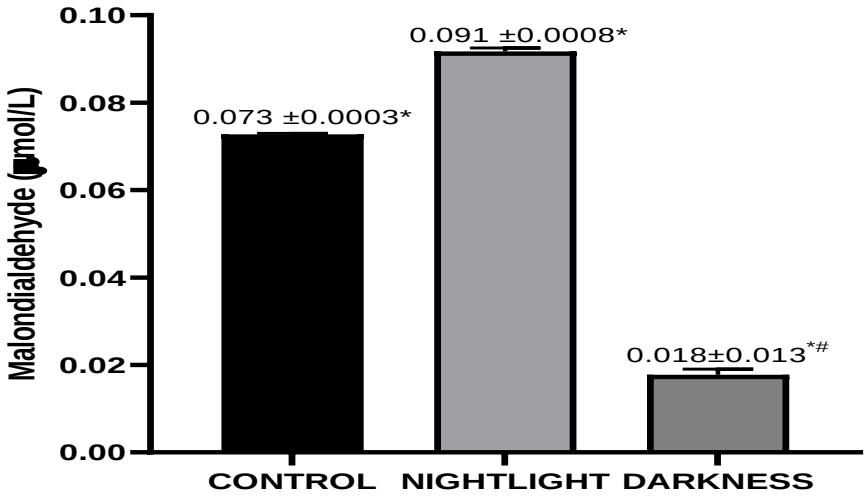

Fig 2: Effect of Nightlight and Darkness exposures on Malondialdehyde in Female Wistar rats Data are expressed as mean ± S.E.M. n=5. (*p<0.05 VS. CONTROL; #p<0.05 VS. NIGHTLIGHT)

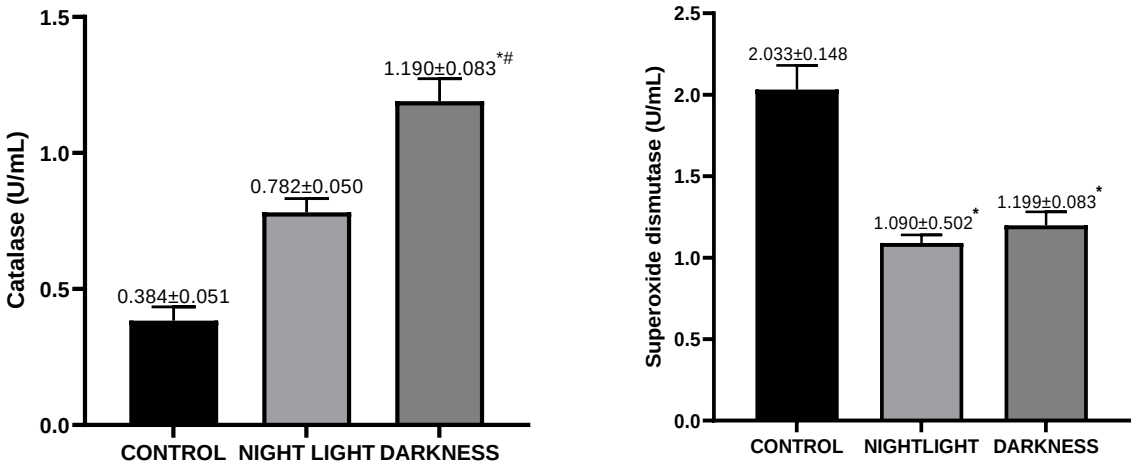

Fig 3: Effect of Nightlight and Darkness exposures on Antioxidant markers in Female Wistar rats
Data are expressed as mean ± S.E.M. n=5. (*p<0.05 VS. CONTROL; #p<0.05 VS.NIGHTLIGHT)

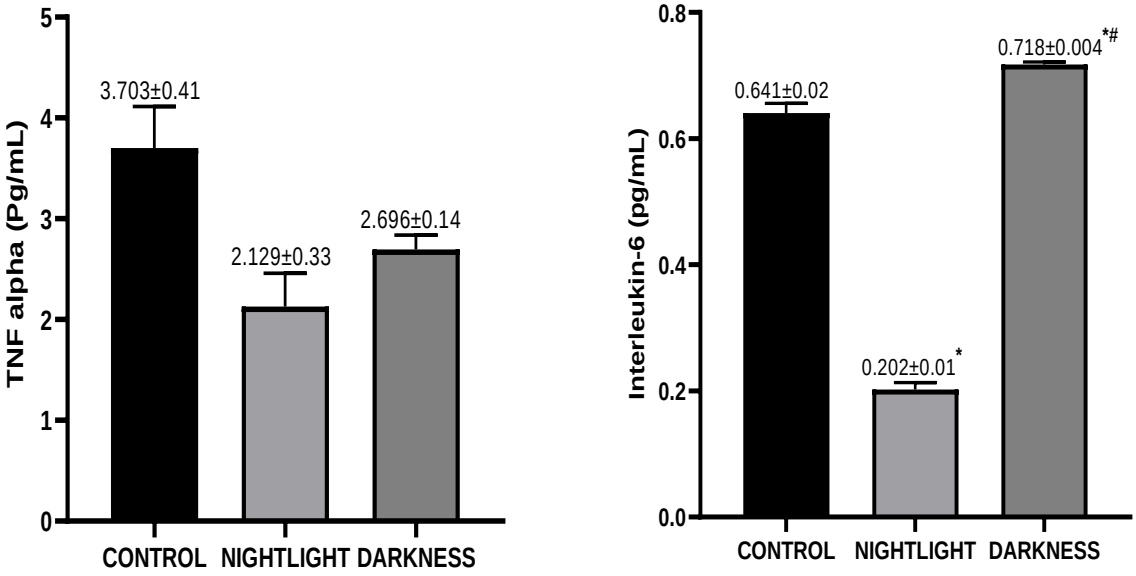

Fig. 4: Effect of Nightlight and Darkness exposures on Inflammatory makers in Female Wistars rats
Data are expressed as mean ± S.E.M. n=5. (*p<0.05 VS. CONTROL; #p<0.05 VS. NIGHTLIGHT)

Table 1: Effect of Nightlight and Darkness exposures on Reproductive Hormones in Female rats

	Control (G-1)	Nightlight G-2)	Darkness (G-3)
Follicle Stimulating Hormone (mlu/ml)	2.246±0.05	2.298±0.06	2.129±0.04
Luteinizing Hormone (mlu/ml)	2.204±0.16	2.641±0.22	2.035±0.03
Progesterone (ng/ml)	2.843±0.15	1.567±0.07*	2.747±0.35#
Estradiol (pg/ml)	19.55±1.46	20.88±0.40	17.51±1.23

Data are expressed as mean ± S.E.M. n=7. (*p<0.05 VS. CONTROL; #p<0.05 VS. NIGHTLIGHT)

DISCUSSION

Light plays a pivotal role in regulating melatonin secretion, and its impact varies depending on various factors, including the time of day, light intensity, spectral properties, and duration of exposure [28,29]. Exposure to light during the nighttime, specifically between midnight and 4 am, coinciding with peak melatonin secretion, completely suppresses melatonin secretion throughout the exposure period [30]. Morning light exposure leads to a phase advance, causing melatonin secretion's peak to occur earlier than usual. Conversely, exposure in the late afternoon, before the core body temperature reaches its nadir, results in a phase delay [31]. The intricate implications for human health have been identified [32], and initial evidence suggests a substantial association between light pollution and severe health issues [33,34], particularly concerning disruptions in circadian rhythms and compromised melatonin secretion, which serves as a marker for these disturbances. This current study investigated and compared the effect of nightlight and constant darkness exposures on plasma melatonin levels, oxidative stress, inflammatory markers, and reproductive hormones in female Wistar rats.

Light exposure initiates a parallel, predominantly unconscious pathway within the visual system, impacting numerous physiological processes including circadian rhythm, sleep/wake cycle, and reproduction [35,36]. Melatonin plays a pivotal role in this pathway, with its production majorly in the pineal gland but also in photoreceptors and the ciliary body epithelium [37,38,39]. The pineal gland and ocular tissues display circadian patterns in melatonin production, releasing the highest levels during periods of darkness and the lowest

levels when exposed to light. This hormone is central to coordinating the circadian system, which serves as the foundation for the biological clock [40]. In this result, the nightlight group had a notable decrease in plasma melatonin levels compared to the control group. Conversely, the darkness group exhibited a significant increase compared to the nightlight group. Light-emitting diode (LED) light sources are used in light bulbs and electronic displays; they provide a sizable amount of short-wavelength light that coincides with the maximal sensitivity of ipRGCs. Studies have reported that LED displays contribute to the suppression of melatonin production at night [5,41]. Under constant darkness, research on fish reported elevated melatonin concentrations in the brain [42]. Studies in other species have also revealed higher melatonin levels in the absence of light [43,44]. Similarly, to this study, an investigation of constant darkness and light in male Wistar rats reported elevated melatonin concentration in the serum under constant darkness and suppression of melatonin secretion with continuous light [26].

In this study, the nightlight group had notably higher plasma MDA levels than the control group. Conversely, the darkness group showed significantly lower levels than both the nightlight and control groups. While plasma CAT did not significantly decrease in the nightlight group, it notably increased in the darkness group compared to both the nightlight and control groups. Additionally, SOD levels significantly rose in the nightlight group compared to the control group, intriguingly, the darkness group also notably decreased compared to the control group. These findings underscore the inhibitory effect of light on melatonin and emphasize melatonin's antioxidant properties. The suppression of melatonin levels due to night light exposure resulted in increased levels of the oxidative stress marker malondialdehyde. Melatonin possesses robust antioxidant effects, employing both direct and indirect mechanisms, rendering it an unparalleled endogenous defender against highly toxic oxygen- and nitrogen-derived free radicals [45]. It directly scavenges reactive oxygen species (ROS) and indirectly enhances endogenous antioxidants and total antioxidant capacity [46]. Its primary modes of action involve free radical scavenging, stimulation of endogenous antioxidative enzymes, and augmentation of other antioxidants. Notably, melatonin, along with its metabolites, which also function as antioxidants, orchestrates an antioxidant cascade, generating radical scavenger products [47], effectively mitigating oxidative damage through various mechanisms [48,49]. Constant light exposure which disrupts circadian rhythm has been demonstrated to induce stress, in turn, stressors play a role in inducing cellular oxidative stress [50]. Faborede et al., reported that constant unpredictable light exposure led to oxidative

stress depicted by increased malondialdehyde levels and reduced SOD, CAT and GSH levels (26). Other studies confirmed this effect under constant light exposure [51,52].

ROS has been demonstrated to enhance TNF-α activities, and reciprocally, TNF-α can trigger additional ROS production [53], resulting in oxidative stress. Additionally, previous observations have revealed that continuous exposure to light amplified the presence of the proinflammatory cytokine TNF-α in rats [51,26]. However, inconsistent with earlier reports, this current investigation displayed no significant differences observed in the proinflammatory marker, plasma TNF-α level across all groups. However, there was a significant decrease in IL-6 level in the nightlight group compared with the control group. Plasma IL-6 levels in the darkness group had a significant increase compared with the nightlight and control groups. Likewise, administering exogenous melatonin before acute conditions has demonstrated its capacity to decrease the inflammatory response and reduce pro-inflammatory cytokines [54]. Moreover, melatonin's performance in mitigating pro-inflammatory cytokines and tissue damage in cells subjected to heightened toxicity and oxidative stress has been confirmed [55,56]. It has been shown to decrease the expression of cytokines, along with reducing apoptosis [57]. El-Missiry et al. noted remarkable amelioration of increased inflammatory cytokines and elevation of IL-10 levels after melatonin treatment in gamma radiation experimental group [58]. Furthermore, Melatonin has been reported to prevent the translocation of NF-kB reducing the upregulation of interleukins and TNF-α [59]. Melatonin significantly lowers TNF- and IL-6 levels, improves CRP levels, and increases redox potential, according to a meta-analysis research [60].

Exposure to light and fluctuations in the concentrations of melatonin are believed to induce anomalies associated with menstrual cycle. Melatonin functions in regulating oxidative stress in the ovarian tissue, and melatonin receptors contribute to controlling FSH and LH secretion [61,62]. Night or shift work, often associated with nighttime light exposure and disruptions in circadian rhythms governing sleep, dietary patterns, and metabolism, has physiological implications. Circadian disruption may affect glucose metabolism, provoke inflammation, and induce oxidative stress, all of which are factors influencing the ovarian cycle [63]. Various studies have reported alterations in reproductive hormone levels among shift workers, including higher estradiol concentrations [64,65]. Additionally, night or shift work has been associated with elevated LH levels throughout the cycle [66], as well as higher FSH and LH levels during the luteal phase [67], as observed in a Seattle-based study. Li et al. also reported detrimental effects on ovarian reproductive functions in rats under constant light and constant darkness conditions

[1]. While this study has limitations, such as the absence of hormone measurements across different phases of the estrous cycle, it's important to note that there were no significant differences observed in gonadotropic and gonadal hormones, plasma FSH, LH, and estradiol, across all groups in the present study. However, there was a significant decrease in progesterone concentrations in the nightlight group against the control, and the darkness group had a significant increase compared with nightlight exposure.

CONCLUSION

Light plays a crucial role in regulating melatonin secretion, with its effects varying based on factors like time of day, light intensity, and exposure duration. Extreme light exposures markedly alter plasma melatonin level. Furthermore, continuous light and dark exposures induce oxidative stress and inflammation. Light pollution disrupts melatonin production and can lead to significant health issues, including disruptions in circadian rhythms and increased oxidative stress.

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