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RESEARCH ARTICLE OPEN ACCESS

The effect of light duration on weight, hormonal parameters and oxidative stress indexes in young white male rats: An experimental study

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Abstract
Objective: Assessing the effects of light exposure at night is crucial because it disrupts melatonin production, a key regulator of circadian rhythms and growth processes. This study aimed to determine how exposure to nighttime lighting affects melatonin and physiological properties in young white male rats.
Method: Fifty-two male rats (20–25 days old, 85–100g) were divided into four groups and treated for 15 days under a 12-hour light/dark cycle. The control group (C) had a normal cycle, while groups T1, T2, and T3 were exposed to continuous light for 4, 8, and 12 hours, respectively. Melatonin, MDA, GPX, and SOD levels were measured. Statistical analysis was performed using SPSS v.25 with significance set at P≤0.05.
Results: Treated groups showed significantly higher body and brain weights compared to the control, though pituitary and thyroid weights showed no significant differences. Melatonin levels dropped significantly in treated groups, while MDA and GPX levels increased. SOD levels were higher in the control group. Brain weights in treatment groups were (1.21±0.039, 0.95±0.004, 1.1±0.95) vs. control (0.96±0.017); melatonin (168.3±7.881, 114.2±4.133, 62.8±1.212) vs. control (189.2±9.815); MDA (3.04±0.037, 3.9±0.065, 4.99±0.353) vs. control (1.9±0.418); GPX (3.07±0.073, 3.9±0.279, 4.5±0.346) vs. control (2.3±0.233); and SOD (1.76±0.256, 1.54±0.133, 1.06±0.153) vs. control (2.01±0.096).
Conclusion: Our findings revealed that exposure to artificial lighting can have adverse effects on our bodies. This includes lowering melatonin levels while increasing body weight and ushering negative impacts on oxidative markers.

Keywords: Melatonin, Effect of light, Weight percentage of the brain, Pituitary gland, Body weight gain.

Plain English Summary

We conducted a study to assess how exposure to nighttime lighting affects melatonin and the performance of young white male rats. We experimented with 52 male rats with an age range of 20- 25 days. The rats were divided into four groups and were treated with the usual 12 hours of daylight and 12 hours of darkness. The control group was exposed to the normal light-dark cycle while the treatment groups were exposed to varying lengths of continuous lighting. Our findings showed that rats in the treatment groups gained more weight than those in the control group. Specifically, the brain weight was considerably higher in the treatment group than in the control. Melatonin levels in the treatment group were significantly lower compared to the control. The comparison of growth hormone between the treatment and control groups was inconclusive. However, the oxidative stress factors, MDA and GPX levels were significantly higher in

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the treatment group. In contrast, the enzyme, superoxide dismutase was higher in the control group. In conclusion, our study found that exposure to artificial lighting can harm our bodies.

Background

Being around artificial light at night can lead to metabolic disorders and an increase in body weight (1). The rate of secretion of melatonin in the blood varies with age and time of the day. For instance, melatonin levels are low in the extremes of life (in infants and the elderly) (2). Melatonin is produced by the pineal gland, which is also referred to as the epiphysis because of its coniferous shape and weight of around 0.1 grams. It is located in the thalamus- adjacent to the third brain ventricle (3, 4). Melatonin (N-acetyl-5-methoxytryptamine) is a chemical that is typically released during the night. This chemical is released according to the authorized daily rhythm and cycle of light and darkness. When the pineal gland is stimulated in the dark, the activity of this gland is inhibited, a path is taken that converts light-related external stimuli into internal stimuli, resulting in the production of melatonin and light particles are absorbed by light-sensitive retinal ganglion cells (ipRGCs) (5).

Melatonin is released into the blood as a neurotransmitter and is transported to all parts of the body (6). Other organs that release melatonin include the ovaries, lymphocytes, retina, bone marrow, digestive system and the thymus. However, the production of melatonin in these organs is not dependent on light but is regulated by the circadian rhythm. Sleep disorders like having trouble sleeping late, and having difficulty falling asleep are both common in society (7). Sleep deprivation at night increases the risk of breast cancer, immune suppression, and numerous diseases associated with night lighting and the associated decrease in melatonin production. The deficiency of melatonin production is linked to the development of multiple diseases, including B. endocrine secretion disorder, neurological diseases, infections, digestive system issues, etc. (8, 9).

The purpose of this research is to assess the effect of exposure to light at night on the hormone melatonin and growth, the cause of weight gain, and the effect on the weight of the brain, pituitary gland, and thyroid gland, as well as the negative effects of light on indicators of oxidative stress. This assessment is crucial because exposure to light at night disrupts melatonin production, a key hormone regulating circadian rhythms, metabolism, and growth (10). Suppressed melatonin can lead to weight gain by altering appetite regulation and energy balance (11). Additionally, changes in light exposure may affect the weight and function of the brain, pituitary, and thyroid glands, impacting endocrine health (12). Excessive light exposure also increases oxidative stress, contributing to cellular damage, ageing, and disease risk (13). Understanding these effects is essential for mitigating health risks associated with artificial light exposure.

Materials and Methods

The experiment included 52 healthy young white males (after weaning), whose ages were between 20 and 25 days, and who weighed 85 to 100 g. They were divided into four groups of 13 animals each (52 total)- (C): Exposed to normal daylight. First treatment group, T1: Animals were subjected to light exposure that was continuous for 4 hours (from 10:00 pm to 2:00 am). Second treatment group, T2: The animals were subjected to continuous light for 8 hours (from 8:00 pm to 4:00 am). Third treatment group, T3: The animals were subjected to continuous light for 12 hours (6:00 pm-6:00 am). Three animals from each experimental group were killed 13 days after the start of the experiment, and the concentrations of melatonin, antioxidants, and the oxidative stress index (MDA) were measured. The remaining animals were killed on the 15th day after the experiment's start to assess the remaining parameters of the experiment.

Body weight gain and Weight of organs

The rate of body weight gain was extracted by measuring the weights of the animals before and after the experiment using a sensitive electronic scale (sensitive electronic scale (Sartorius Meter 200 AE, Germany), which was calibrated daily to determine the weight differences that accompanied the effect of periods of exposure to lighting. The initial weight rate was subtracted from the final weight rate. After removing the brain, pituitary gland, and thyroid gland, and removing the tissues and fatty parts surrounding them, these organs were weighed using a sensitive electronic scale, and the weights were recorded in relation to the animal's body weight (mg/100 g of body weight). The rats were sacrificed by ketamine xylazine injection.

A hormonal assay using the ELISA Technique

The concentrations of serum Melatonin (MT) and Growth Hormone (GH) were determined as described by the manufacturers (Sola Biotechnology Co., Ltd., Beijing, China and

Shanghai Biotechnology Co., Ltd., Shanghai, China).

Measurement of oxidative stress

The degree of oxidative stress was determined by enzyme-linked immunosorbent assay (ELISA) according to the instructions of the ELISA kit (Nanjing Jiancheng Biotechnology Co., Ltd.). The variables associated with oxidative stress, MDA, SOD, and GPX, were determined using an ultraviolet spectrophotometer at an absorption frequency of 450nm, and the level of expression was calculated.

Statistical analysis

Treatments and control groups were statistically compared using ANOVA one-way and LSD at a 5% probability level.

Results

Melatonin concentration and oxidative stress indicators in blood serum

Table 1 illustrates the effects of different photoperiods on melatonin levels and markers of oxidative stress. The melatonin levels of the first, second, and third treatment groups were significantly lower than the normal level recorded in the control group (with a probability of 5%). Findings showed that the concentrations of MDA and GPX in the first, second, and third treatments were significantly higher than the control group (with a probability of 5%). Conversely, the statistical analysis results demonstrated that the SOD concentration in the second and third treatment group was significantly lower than the control group. However, the first treatment group failed to achieve the critical threshold ($p<0.05$).

Table 1: The effects of different light periods on melatonin concentrations and markers of oxidative stress 13 days after the experiment's start

Parameters Group	MDA	GPX	SOD	Mt
C	1.9±0.418 a	2.3±0.233 d	2.01±0.096 a	189.2±9.815 a
T1	3.04±0.037 c	3.07±0.073 c	1.76±0.256 a	168.3±7.881 b
T2	3.9±0.065 b	3.9±0.279 b	1.54±0.133 ab	114.2±4.133 c
T3	4.99±0.353 b	4.5±0.346 a	1.06±0.153 b	62.8±1.212 d
LSD	0.71	0.82	0.55	21.69

Small different letters within each group indicate significant differences at ($P < 0.05$)

The rate of body weight increase and the percentage of brain, pituitary, and thyroid mass

The findings in Table 2 showed that various increases were significant (at a 5% probability level) in the weight gain of animals in the treatment groups compared to the control group. Regarding the percentage of the brain's weight, our findings showed a significant increase (at a probability level of 5%) in the first treatment group compared to the control group and the second treatment group. The results showed a significant increase in the first group compared with the second group and control while there was no significant difference between the first and third groups at a probability level of 5%. The slight increases in the weight percentages of the thyroid and pituitary glands in the treatment groups were not significant compared ($P > 0.05$) with the control group.

Table 2: The effects of different photoperiods on the rate of weight gain and the proportion of weight in the brain, the pituitary gland and the thyroid gland

Parameters Groups	%Weight percentage of the pituitary gland	The % Weight percentage of the thyroid gland	The weight percentage of the brain%	Weight gain%
C	0.0036±0.0004 a	0.0045±0.0006 A	0.96±0.017 b c	32.3±0.278 C
T1	0.0025±0.0004 a	0.0056±0.0001 A	1.21±0.039 A	36.2±0.200 B

T2	0.0034±0.0005	0.0047±0.00015	0.95±0.004	49.5±0.166
	a	A	C	A
T3	0.0027±0.0003	0.0052±0.0001	1.1±0.95	39.7±0.217
	a	A	Ab	D
LSD	0.0012	0.00014	0.146	0.632

Different letters between any two members of the group indicate significant differences at a P value of 0.05 or less.

Melatonin and growth hormones

The data in Table 3 indicate that the melatonin levels of the treated animals were significantly lower than the controls ($p<0.05$). For growth hormones, the change in the concentration of these hormones was minimal. The decrease was not significant in either the treatments or the results.

Table 3: The effects of different photoperiods on melatonin and growth hormones are different

Parameters Groups	GH ng/ml	MT ml/pg
C	45.18±1.574	192.02±7.24503
	A	A
T1	±1.30145.11	167.4±6.20967
	A	B
T2	±1.8744.9	110.56±3.26766
	A	C
T3	±1.39744.8	64.82±3.46569
	A	D
LSD	4.65	19.99

Different letters between any two members of the group indicate significant differences at a P value of 0.05 or less.

The index of oxidation

The data in Table 4 which shows the results 15 days after the start of the experiment indicate that the concentrations of MDA and GPX in the first, second and third treatments were significantly higher than the control group (p. 5%). Conversely, the statistical analysis (using the SSP V. 25 program with a probability of 5%) results in this table demonstrate that the concentrations of SOD were notably higher in the control group than in the second and third treatment groups. In contrast, the first treatment group failed to reach the significant threshold ($p>0$ .05).

Table 4 The effect of different lighting durations on oxidation indicators after 15 days of the experiment

Parameters Groups	MDA NMOL / ML	GPX U / ML	SOD U / ML
C	1.56±0.045	2.21±0.093	2.05±0.094
	D	D	A
T1	3.07±0.049	2.98±0.044	1.91±0.106
	C	C	A
T2	4.12±0.122	3.94±0.185	1.23±0.058
	B	B	Bc
T3	4.97±0.051	5.04±0.084	0.99±0.039
	A	A	C
LSD	0.222	0.341	0.234

Different letters between any two members of the group indicate significant differences at P 5%

Discussion

The results of this investigation showed that the total weight of animals exposed to light at night was significantly greater than that of the control group. Exposure to light during the night impairs the basic mechanism of circadianism at the gene and protein levels, leading to metabolic disturbance and body weight gain (14). This is because eating late at night leads to inconsistent eating patterns, which may lead to metabolic problems (15). The current results demonstrated that the brain weight decreased significantly in the treatment group exposed to artificial lighting compared to the control group (32.3±0.278). The process by which environmental lighting conditions affect the brain is not fully understood, but nighttime exposure to light leads to the destruction of the circadian rhythm and the death of the Purkinje cells or neurons in the cerebellum and the death of Purkinje cells in animals before puberty. This occurs because night lighting impairs the growth of the cerebellum and also the weight of the brain in young animals. Exposure to artificial light during the night also impairs the development of the brain but causes body weight loss in humans (16).

The effect on the human brain's weight and the weights of the thyroid and pituitary glands increased marginally in the treated group compared to the control group, but there was no significant difference. For the pituitary gland, their results were consistent with those of Enemali et al (17). Despite the secretory dysfunction, no pituitary changes were observed when pre-pubertal male albino rats were exposed to light for four weeks. No significant alterations were observed, only a slight increase in the weight of the thyroid (18).

The results demonstrated a significant drop in the levels of melatonin in the treated group compared to the control group. Our findings align with findings from other studies. Exposure to artificial light at night causes a 75% reduction in melatonin production. In addition, the circadian system and sleep cycle are disrupted, causing the biological clock to function differently (19). Light at night has a significant effect on the size of the pineal organelles, as the light causes changes in the size and number of these structures and it leads to changes in the intracellular environment that affect the RER and the size of the cisternae, as well as increased concentrations of enzymes in the lysosomes. The Golgi apparatus also changes, although the general structure of the cell and gland is usually 12/12 h of light and darkness (20). Exposure to light late-night indoors inhibits melatonin production. This results in a decrease in the level of melatonin before sleep- the mechanism by which light affects organ functions is related to the eye's perception of light -and this causes a decrease in the level of melatonin. This indirectly affects the production of melatonin and disturbs the circadian rhythm (21).

Regarding growth hormone, the current study did not show a significant difference in growth hormone levels in the treatment group compared to the control group, but the hormone levels remained lower than normal. This may be attributed to the fact that the length of time spent studying with artificial night light was not sufficient to prevent the secretion of growth hormones. In previous research, the average concentration of growth hormones in all animals exposed to light for 35 days was low. This is due to the unregulated nature of the circadian changes and dysfunction of the pineal gland, which may prevent the production of growth hormone releasing factor, which reduces the synthesis and release of growth hormone (22).

The results demonstrated that the concentrations of oxidation markers such as GPX and MDA which are (4.97±0.051) and (5.04±0.084) in the serum of animal groups exposed to different light intensities significantly increased compared to the control group. This occurred due to increased oxidative stress from lipid peroxidation in the mouse brain through the use of light during the night. Additionally, oxidative stress leads to a decrease in melatonin, the brain's natural defence mechanism, causing an increase in GPX to relieve stress (23). This demonstrated that the SOD content (2.05±0.094) in the three light exposure groups was significantly lower than the control group. Our research shows that the decrease in melatonin hormones is likely an important reason for the decrease in SOD levels, because exposure to light during the night directly affects the rhythm of antioxidant enzymes, and SOD levels are also lower in the presence of light. Because SOD levels depend on the level of melatonin secretion, this chemical is released during the middle of the day, in the dark, or at night.

Conclusion

The current study demonstrated that exposure to light at night results in a reduction in the concentration of the melatonin hormone an increase in body weight and brain weight and an elevation in oxidative stress markers.

List of Abbreviations

MDA: Malondialdehyde
GPX: Glutathione peroxidase
MT: Melatonin
GH: Growth hormone

ELISA: Enzyme-linked immunosorbent assay
SOD: Superoxide dismutase

Declarations

Ethical approval and consent to participate
This research was sanctioned by the Scientific Research Ethics Committee of the Department of Biology, Faculty of Education, Qadisiyah University. All experiments were performed following international best ethical practices.

Consent for publication
All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials
The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Author contributions
ABS and AJK were responsible for all aspects of the research, including conceptualization, data collection, laboratory analysis, and manuscript draft. The authors also confirmed the final version and assented to the manuscript's publication.

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