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Impact of continuous light stress on growth performance, organ weight, serum melatonin concentration, and thiol-disulfide homeostasis in rats

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ABSTRACT: This study aims to assess the effects of continuous light stress on the growth performance, live weight gain, specific organ weights, serum melatonin concentration, and modulation of thiol-disulfide homeostasis in rats. The control group (G1, $n = 7$) with 12-hour light/12-hour dark and the experimental group (G2, $n = 7$) with 12-hour light/12-hour light were treated for 12 days. Both groups were weighed daily throughout the study. At the end of the experiment, rats were euthanized after being anesthetized. Blood and organ samples were then collected. Continuous light stress decelerated live weight gain from the 4th day with a pronounced effect observed from the 10th day onwards. Serum melatonin concentration decreased with continuous light exposure with a more significant reduction in G2 group. Analysis of alterations in thiol-disulfide homeostasis revealed a decrease in native thiol and total thiol levels and an increase in disulfide levels in rats exposed to continuous light stress. Additionally, the disulfide/native thiol ratio and disulfide/total thiol ratio increased, while the native thiol/total thiol ratio decreased. A robust and positive correlation was observed between melatonin concentration, body weight, and native thiol ($r = 0.71$, $p = 0.005$). These findings highlight the effects of continuous light exposure on the metabolic balance, circadian rhythms, and cellular redox balance in biological systems.

KEYWORDS: antioxidant, circadian rhythm, light stress, melatonin, thiol-disulfide homeostasis, weight gain

INTRODUCTION

Uninterrupted exposure to intense illumination has been shown to affect fundamental biological rhythms, including the sleep-wake cycle, water consumption, locomotion, blood pressure, heart rate, and body temperature. These rhythms are regulated by the intricate mechanisms of the suprachiasmatic nucleus within the brain. Studies show that residing under constant light conditions not only increases cortisol levels in both male and female rats but also serves as a model for inducing chronic stress [1, 2].

The extended exposure to constant light has been observed to disturb the synchronicity of biological rhythms in adult rats, affecting impacting crucial aspects such as motor activity, body temperature, melatonin levels, and sexual hormones. Interestingly, a significant difference emerges when considering albino rats exposed to constant light during their lactation period [3]. Light during the neonatal period is critical. These initial exposures to light establish the foundation for the cohesive operation of biological rhythms in mammals, exerting a substantial impact on their capacity to adapt to the complexities of their adult environments. Hence, careful attention to lighting during these developmental stages remains of paramount importance, as it shapes lasting effects upon the circadian systems of mammals, ultimately enhancing their resilience in the face of forthcoming environmental challenges [1, 4]. Melatonin, a hormone produced and released by the pineal gland, plays a crucial role in regulating daily rhythms across various vertebrates, including rodents and humans. This hormone exhibits a distinct daily pattern in its levels, predominantly released during the nighttime hours. Interestingly, this pattern remains consistent among species, regardless of their diurnal or nocturnal activity. For example, in rodents, melatonin production peaks during their active nighttime phase, while in humans, the highest melatonin levels align with the hours of sleep-in darkness [5, 6]. Melatonin functions as a versatile antioxidant, effectively countering free radicals by directly neutralizing them. Its unique combination of high lipophilicity and hydrophilicity enables it to pass through cell membranes with ease. Naturally present in cells and obtained from sources such as fruits and vegetables, melatonin maintains a significant presence within our bodies. Despite varying levels in human serum and cerebrospinal fluid, its toxicity remains minimal. Unlike typical antioxidants, melatonin does not engage in redox cycling. Instead, it functions as a terminal antioxidant, undergoing molecular rearrangement to eliminate free electrons. This distinctive process generates multiple byproducts from a single melatonin molecule, enabling it to combat a wide range of reactive oxygen species (ROS) — distinguishing it from traditional antioxidants that target fewer ROS. Melatonin outperforms common antioxi-

dants like ascorbate and α-tocopherol in transforming radicals and effectively reducing damaged DNA. Its extensive distribution within cells enhances the protection of lipids, proteins, and nuclear DNA from oxidative damage. Furthermore, melatonin superiority over its analogues emphasizes how the structural attributes of its indole moiety govern its reactivity and effectiveness [5, 7].

Thiol-disulfide homeostasis has attracted increasing attention in recent years, contributing to a more comprehensive understanding of its role in various medical conditions. Dysregulated thiol-disulfide homeostasis has been implicated in numerous disorders with elusive origins. Emerging evidence sheds light on thiol-disulfide homeostasis involvement in a wide spectrum of diseases. Studies reveal alterations in biomolecule levels, including native thiol, total thiol, and disulfide bonds. These alterations may reveal pathogenic mechanisms, serving as diagnostic, prognostic, and therapeutic markers. In the field of cardiac pathologies, investigations have established links between thiol-disulfide homeostasis and the severity of coronary atherosclerosis. Endocrine disorders such as prediabetes, type 1 and type 2 diabetes mellitus exhibit shifts in native thiol, total thiol, and disulfide levels. Similarly, neurological diseases, psychiatric conditions, respiratory disorders, gastrointestinal ailments, and others manifest intricate correlations with thiol-disulfide homeostasis [8–10].

This study aims to investigate the combined effects of continuous light exposure on growth performance, organ weights, serum melatonin concentration, and thiol-disulfide homeostasis in rats. A comprehensive literature review revealed a gap in knowledge regarding the simultaneous evaluation of these parameters in the context of continuous light-induced stress. Therefore, this study presents a novel exploration of the hitherto uninvestigated influence of continuous light on the thiol-disulfide homeostasis system in rats.

METHODOLOGY

Fourteen female Wistar albino rats, each weighing between 250 and 260 g, were included in the study. Following a 10-day adaptation period, the rats were kept in an environment with a temperature of 23 ± 1 °C and humidity of 50% ± 5. The groups were provided with commercial pellet feed (Bayramoğlu Yem®, Erzurum, Türkiye, Table S1) and *ad libitum* access to drinking water.

The rats were divided into 2 groups with balanced body weights, and pellet diets were placed on the cage floor to avoid shading. The first group (Control, G1, $n = 7$) was maintained in a 12-hour light/12-hour dark environment for 12 days. The second group (G2, $n = 7$) was housed in a 12-hour light/12-hour light environment for 12 days. Rats were weighed daily throughout the 12-day period.

After intramuscular injection of xylazine (15 mg/kg, Rompun®, Bayer, Germany) and ketamine hydrochloride (75 mg/kg, Ketasol®, Richter Pharma AG, Austria), euthanasia was performed on the rats by the cervical dislocation method. Subsequently, blood was collected, and organ weights were measured. The heart, kidneys, and liver were immediately separated from external tissues and weighed.

Blood samples were collected using gel-vacuum tubes (BD Vacutainer® SST II Advance, Becton, Dickinson and Company, UK) via cardiac puncture and then centrifuged at 1,200g for 10 min (NF 400R®, Nüve, Türkiye). The obtained sera were stored at −18 °C until biochemical measurements were performed.

The serum melatonin concentration was determined using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit specifically designed for rats (Bioassay Technology Laboratory, China).

Native thiol and total thiol measurements were conducted using commercial spectrophotometric kits, namely the Native Thiol Assay Kit and Total Thiol Assay Kit (Rel Assay Diagnostics®, Mega Tıp, Türkiye). This procedure was carried out in accordance with the specified kit's protocol. Additionally, utilizing the obtained native thiol and total thiol data, the values for disulfide, disulfide/native thiol ratio (%), disulfide/total thiol ratio (%), and native thiol/total thiol ratio (%) were calculated [11].

G-Power 3.1.9.7 was used to do power analysis before the trial. Based on the analysis, the sample size was determined with an effect size (d) of 1.88, test power of 0.95, significant level of 0.05, and findings from the study of Semenovich et al [12]. In the study, growth performance data, organ weights, and biochemical measurements were presented as mean ± standard deviation (SD). The normal distribution of data within groups was confirmed through the Shapiro-Wilk test. For the analysis of growth performance and live weight gain data over days, two-way analysis of variance (ANOVA) was employed. Statistical analysis involved the examination of group effects, time effects, and group x time interactions, with Tukey's multiple comparison test used for pairwise comparisons between days. Following an assessment of variance homogeneity using the Levene test, group differences in organ weights and biochemical analysis results were compared using the independent sample T-test. Statistical analyses were conducted using GraphPad Prism® (Version 9.5.1, GraphPad Software Inc., San Diego, CA, USA), and any obtained statistical differences were deemed significant at the $p < 0.05$ level.

RESULTS

Throughout the study, continuous light stress decelerated live weight gain from the 4th day onwards and diminished it in subsequent periods. At the completion

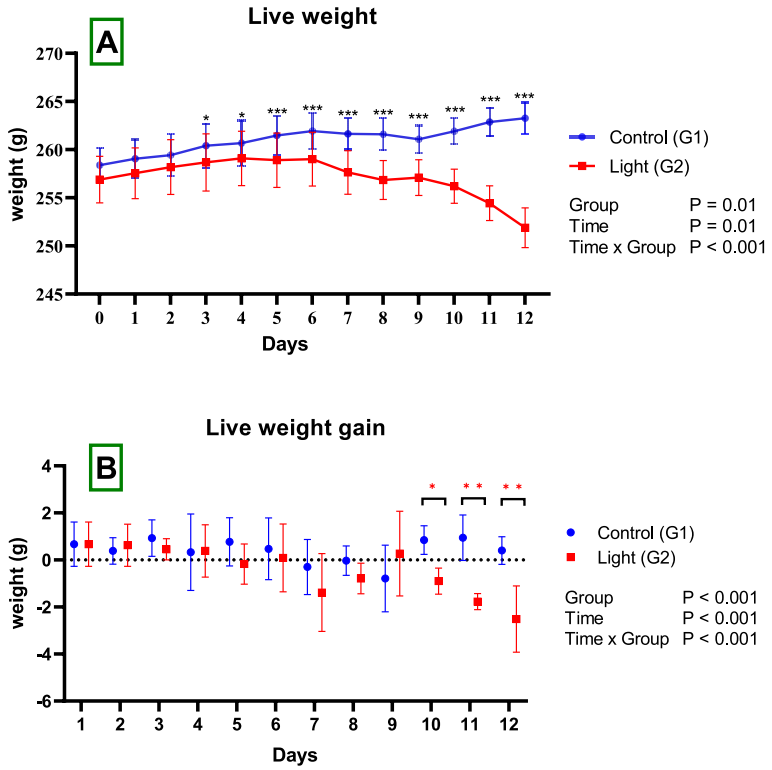

Fig. 1 A: Changes in growth performance in rats throughout the study. B: Changes in live weight gain in rats over the study duration. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

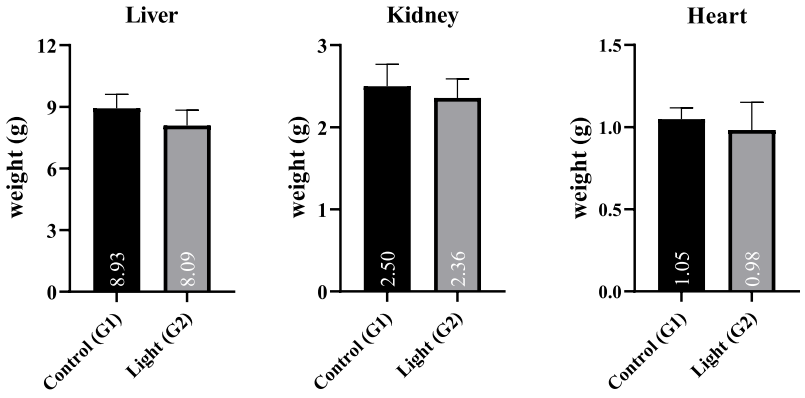

Fig. 2 Changes in liver, kidney, and heart weights of rats according to groups.

of the study, the live weight of G1 was found to be significantly higher compared to that of G2 ($p < 0.001$). Moreover, the group effect was statistically significant throughout the study ($p = 0.01$), and from the 4th day onwards, statistically significant differences in live weight between the 2 groups were observed until the end of the study (Fig. 1A). Regarding live weight gain, the effects of continuous light stress were determined to be statistically significant from the 10th day onwards. A decrease in live weight and live weight gain was observed in G2 compared to G1 (Fig. 1A,B). The group effect, time effect, and group x time interaction were all found to be statistically significant for live weight gain ($p < 0.001$, Fig. 1B).

In rats exposed to continuous light, the weights of the liver, kidney, and heart were numerically different compared to the control group; however, there was no statistically significant difference (Fig. 2).

The serum melatonin concentration decreased following 12 days of continuous light exposure. The concentration of serum melatonin in G2 exhibited a statistically significant decrease ($p < 0.01$) compared

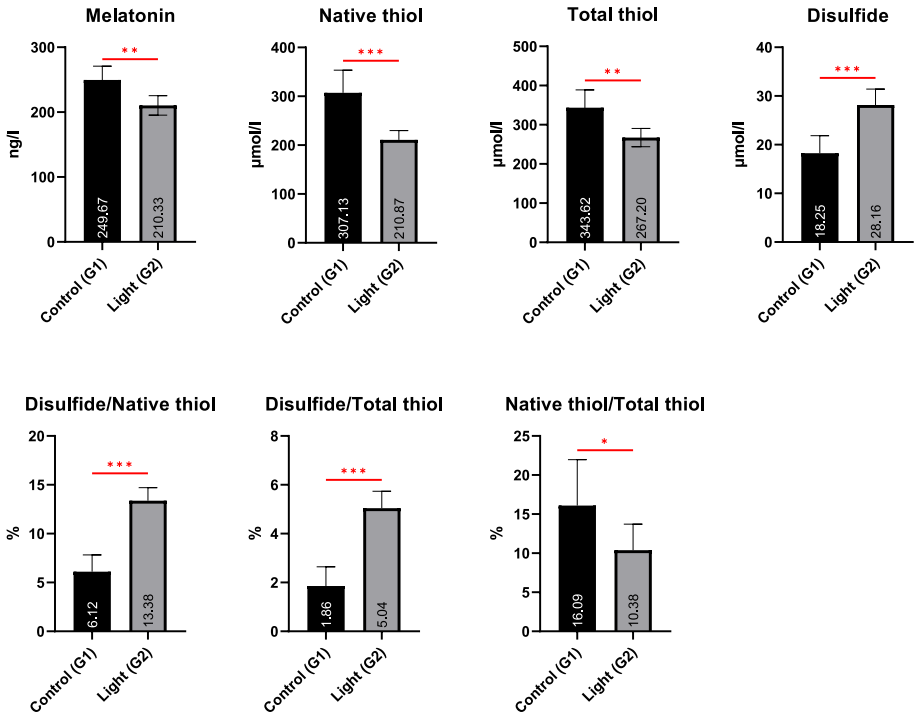

Fig. 3 Serum melatonin concentration and thiol-disulfide haemostasis in rats exposed to continuous light for 12 days. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

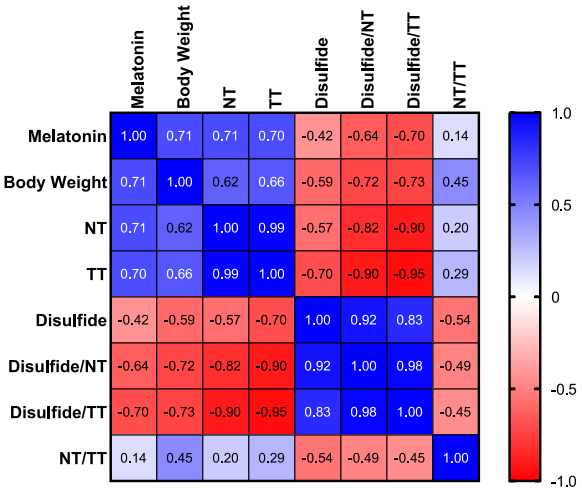

Fig. 4 Correlation coefficients between body weight, melatonin, and thiol-disulfide haemostasis in rats. NT: Native thiol and TT: Total thiol.

to G1 (Fig. 3).

Continuous light stress on rats resulted in a reduction in native thiol ($p < 0.001$) and total thiol ($p < 0.01$), while disulfide ($p < 0.001$) increased. Additionally, in G2, the disulfide/native thiol ratio ($p < 0.001$) and disulfide/total thiol ratio ($p < 0.001$) increased, while the native thiol/total thiol ratio ($p < 0.05$) decreased compared to G1 (Fig. 3).

In our study, Pearson correlation coefficients related to body weight, melatonin, and thiol-disulfide haemostasis are presented in Fig. 4. A strong and positive relationship was observed between melatonin concentration and body weight, as well as native thiol ($r = 0.71$, $p < 0.01$). The correlation between total thiol levels and melatonin was also found to be significantly positive ($r = 0.70$, $p < 0.01$). On the other hand, a negative correlation was identified between melatonin and disulfide/native thiol ratio ($r = -0.64$, $p < 0.05$). Additionally, a significant negative correlation was observed between melatonin and disulfide/-total thiol ratio ($r = -0.70$, $p < 0.01$).

DISCUSSION

In our study, we investigated the dynamics of growth performance, live weight gain, serum melatonin concentration, and thiol-disulfide haemostasis in rats exposed to continuous light for a duration of 12 days. Despite previous research [13, 14] focusing on the impact of continuous light exposure on growth performance and serum melatonin concentration in rats, there has been a notable absence of studies examining the changes in thiol-disulfide haemostasis. Therefore, our study may represent the first exploration of the effect of continuous light stress on thiol-disulfide haemostasis in rats.

In rats exposed to continuous lighting, there are in-

dications of a potential decline in both food and water intake. Specifically, studies have shown that exposure to constant light for 24 h can suppress food intake [15]. Moreover, short-term exposure to continuous light has been suggested to have adverse effects on mouse or rat body weight [16, 17]. However, conflicting findings exist, with a study [14] arguing that continuous light does not impact body weight. In our study, we observed a negative impact of continuous light exposure on the growth performance and live weight gain in rats. These reductions in weight gain are believed to be linked to the diminished consumption of food and water due to continuous light exposure.

After the onset of darkness, melatonin secretion in rats experiences a surge, reaching its peak in the middle of the night and declining in the second half. This inherent rhythm persists in continuous darkness. However, continuous exposure to light disrupts the daily rhythm of N-acetyltransferase, a pivotal enzyme in melatonin synthesis [18]. Despite this, continuous exposure to light conditions can lead to an average sixfold decrease in plasma melatonin levels [19]. In line with previous findings [15, 20], our study also corroborates a reduction in serum melatonin concentration resulting from continuous light exposure.

Stress, commonly defined as the generalized response of an organism to internal and/or external threats known as stressors, plays a vital role in maintaining homeostasis [21]. The timely and appropriate response of living organisms to stress factors is imperative for sustaining homeostasis. This stress response is primarily orchestrated through the release of catecholamines and corticosteroids, complemented by the secretion of various hormones such as vasopressin and oxytocin [22]. Stressors, whether psychological or physiological, uniformly impact the stress system, eliciting analogous physical and behavioural responses in the form of general adaptation or the stress syndrome [23]. Prolonged exposure to light is well-established to disrupt circadian activity, influencing physiological and primary metabolic processes [24]. Concurrently, continuous light exposure induces agonistic behaviours that disrupt group cohesion in certain individuals [16]. The ensuing disrupted physiological activities and agonistic behaviours, coupled with intragroup unrest, collectively induce stress, and a prominent manifestation is the elevation of corticosterone concentration [16, 23]. In the scope of our study, rats subjected to 12 days of continuous light exposure exhibited an initial deceleration in body weight gain, followed by a subsequent decrease. These observed phenomena may indicate the onset of stress in rats. Recently, we investigated alterations in thiol-disulfide homeostasis, a metric of oxidative stress extensively measured in various diseases and acute/chronic stress conditions [9]. Our findings indicated that continuous light exposure in rats resulted in decreased native and total thiol levels and increased disulfide values. Twelve days of continuous light stress were observed to induce dynamic changes in thiol-disulfide homeostasis in rats. Additionally, the ratios of disulfide/native thiol and disulfide/total thiol were identified as potential stress indicators. In a parallel study involving rats, chronic cold stress was found to decrease native and total thiol, whereas acute cold stress increased disulfide levels [23].

A study comparing patients with traumatic stress disorder to healthy controls revealed a shift in thiol/disulfide homeostasis towards disulfide [25]. Another investigation noted lower native thiol levels but higher disulfide levels and disulfide/native thiol ratios in patients with general anxiety disorder and panic disorder compared to healthy controls [26]. Our study further observed that continuous light stress in rats reduced antioxidant capacity and increased oxidative stress, consequently leading to an augmentation of thiol/disulfide homeostasis towards disulfide.

CONCLUSION

This study marks a pivotal stride in evaluating the profound impacts of continuous light stress on biological systems. The observed reduction in live weight underscores the rapid disruption of thiol-disulfide homeostasis in organisms subjected to continuous light, emphasizing the critical role of light in metabolic equilibrium. Additionally, the significant decrease in melatonin levels illustrates how continuous light can suppress circadian rhythms, influencing the organism internal clock. Unveiling that continuous light can induce cellular damage by diminishing antioxidant capacity and heightening oxidative stress fills a substantial void in this field.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.113.

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Appendix A. Supplementary data

Table S1 The content of pellet feed.

Analytical (%)		Additive		Trace element component (mg/kg)	
Humidity	12.80	Vitamin A	12,000,000 IU/kg	Manganese (manganese sulfate)	86
Crude protein	23.00			Iron (iron sulfate monohydrate)	31
Crude oil	3.70			Zinc (zinc oxide)	96
Raw cellulose	7.70			Cobalt (cobalt carbonate)	0.50
Raw clay	6.90			Selenium (selenium selenite)	0.30
Sodium	0.50			Iodine (calcium iodate anhydride)	2.20