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The effects of different light spectrum on some oxidative stress parameters in male and female rats

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Abstract

The objective of this study was to investigate oxidative stress parameters in plasma, liver, and brain of Sprague Dawley rats grown under a compact fluorescent lamp with purple, blue, green, yellow, orange, red, and white. For this purpose, 56 male and 56 female rats were housed in standard cages under seven different light colors from weaning until puberty. Malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GPx) in plasma, liver, and brain were examined as oxidative stress parameters. Two factors which are gender and light were investigated regarding the main effects by Two-way ANOVA with the General Linear Model procedure. The different light spectrums had a significant impact on MDA, GSH and, GPx of plasma, liver, and brain ($P<0.05$). The red group resulted the highest in plasma, liver, and brain MDA. In addition, some of the examined parameters were different for the gender basis. The plasma GSH, the liver MDA, the liver and brain GPx were different in male and female rats ($P<0.05$). Only the liver GPx enzyme activity was higher in male rats, the other parameters in the female rats had a higher value. In conclusion, low-wavelength lights may be more useful for rats' environments when compared to high wavelengths in manner oxidative stress. However, illumination is not the sole phenomenon for raising animals. Environmental demands may vary from time to time and even according to gender. Moreover, it may be useful for studies to be conducted by considering other lighting factors such as intensity and cycle.

Keywords: Light Spectrum; Lighting; Rat; Oxidative Stress

1. Introduction

Light is an important macro-environment factor for organisms. Light significantly affects organisms for the ordinary course of growth and development, muscle and bone development, immune system, hormone and enzyme systems, and daily activities. Therefore, lighting design should be optimally balanced in animal houses the maintain an optimal environment [1,2]. Light is a special form of energy that has a certain energy and frequency, moves in the form of waves, and is explained by particle effect, wave, and photon theories. It can also be defined as the propagation of very small atomic particles in the form of waves. Electromagnetic radiations of different shapes all travel in space as energy waves. The region between the two waves formed is called the wavelength. Since the wavelength ranges from 380 nm to 780 nm infinitely, there are an infinite number of colors from purple to red. The smallest wavelengths of the visible spectrum correspond to purple, and the largest wavelengths to red. Visible electromagnetic waves are visible light waves. In healthy people, the eye can perceive between 380 and 780 nm [3].

Light greatly affects the appearance, behavior, and physiology of rats. The effect of light on rats is much greater than its effects on the eyes. Light, which is the most important factor of circadian rhythm, is necessary for daily life, endocrine

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levels and metabolism of rats. Many factors can affect the light needs of rats. Some of these; light intensity and wavelength, exposure time of rats to light, pigmentation of rats, body temperature, hormonal status, age, species, sex, and location of the cage [4]. Many studies in rats have demonstrated the physiological importance of photosensitive retinal ganglion cells (different from rod and cone cells) for neuroendocrine, circadian, and neurobehavioral regulation. According to the origin of light, lighting is divided into two natural and artificial lighting. Lighting should be evenly distributed over the area where the rats are located and should provide adequate lighting for the welfare of the animals. Another essential factor of lighting for rats is the photoperiod. The light/dark cycle regulates the circadian rhythm and reproductive cycle in rats. It is appropriate to arrange the length as 12/12 or 10/14 (light/dark) for rats for a day.

Oxidative stress occurs when disequilibrium between the amount of oxidants and antioxidants in organisms. This imbalance leads to tissue damage. On the other hand, the retina is susceptible to oxidative stress due to carrying out the capture of light photons and consistently interacting with the light [5]. The photo transduction cascade is activated by light and stimulates photoreceptor cells which promotes hyperpolarization. Light overexposure might induce the secretion of melatonin and desynchronization of the circadian rhythm [6].

In this context, the objective of this study was to investigate oxidative stress parameters in plasma, liver, and brain of Sprague Dawley rats grown under a compact fluorescent lamp with purple, blue, green, yellow, orange, red, and white.

2. Material and methods

2.1. Experimental design and light conditions

A permit was obtained from the Animal Experiments Local Ethics Committee of Adiyaman University with the decision numbered 2021/002 for the conduct of the research. The study was conducted at the Experimental Animal Production Application and Research Center at Adiyaman University. The seven experimental groups were formed as purple, blue, green, yellow, orange, red, and white lighting groups. Rats were housed in conventional rat cages and the lighting intensity was designed to be equal in all groups in the cage, in the range of 30-50 lux (the 30 lux under the feeders and drinkers, the 50 lux as they approached the light source). The cages were placed in the dark room and the lights belonging to the research groups were prevented from affecting each other. The light/dark period is arranged to be the 12/12 hours. In the study, the power analysis was applied to determine the sample size. A total of 56 female/56 male Sprague Dawley rats were used in the study, 8 female and 8 male rats for each light group. Rats of similar weight and age were used in all experimental groups. Males and females were housed in separate cages for 5 days, allowing them acclimatization to the lighting. The experiment lasted from weaning until puberty.

2.2. Tissue preparation

At the end of the experiment, 8 male and 8 female rats from each group were euthanized under anesthesia, and blood, liver, and brain tissues were collected. The tissues were homogenized 1/9 (w/v) with phosphate buffer and centrifuged at 3200 g. After centrifuge, the separated supernatants were stored in 1.5 mL tubes at -80 °C until analyzed.

2.3. Determination of MDA

The measurement of MDA, a product of lipid peroxidation, was made according to Uchiyama and Mihara [7]. The 250 µl of supernatants were added into 2.5 mL of color reagent, and vortexed. This mixture was heated in 95 °C bath for 30 min. The samples were immediately removed from the hot water bath at the end of 30 min, placed into a cold water container, cooled, and centrifuged at 3200 g for 5 min. The supernatants were taken into glass cuvettes and read against a blank with distilled water at 532 nm in a spectrophotometer (SHIMADZU 1280, Japan). The absorbance of the pink-red color formed as a result of the reaction of MDA with thiobarbituric acid at 95 °C was evaluated spectrophotometrically.

2.4. Determination of GSH and GPx enzyme activity

GSH level was determined according to Moron et al. [8]. The 0.5 mL sample was taken and 0.4 mL 10% Trichloroacetic acid was added to it and vortexed, then centrifuged at 3200 g for 5 min. The 0.5 mL of the obtained supernatants were taken into separate test tubes, and 0.1 mL of Dithiobis (2-nitrobenzoic acid) solution and 2 mL of tris buffer were added and vortexed. The resulting mixture was placed into glass cuvettes and read against a distilled water blank at 412 nm in a spectrophotometer (SHIMADZU 1280, Japan). The GPx enzyme activity was determined by measuring at 412 nm on a spectrophotometer (SHIMADZU 1280, Japan) using Lawrance and Burk methods [9].

2.5. Statistical analysis

All the data were analyzed with IBM®SPSS version 20 software. Two factors which are gender and light were investigated regarding the main effects by Two-Way ANOVA with the General Linear Model procedure. The data were presented as mean and standard error mean. The statistical significance was considered when P≤0.05 with a 95% confidence level.

3. Results

The effect of light spectrum on some oxidative stress parameters in plasma, liver and brain tissues of rats is presented in Table 1. The different light spectrums (Purple, Blue, Green, Yellow, Orange, Red and, White) had a significant impact on MDA, GSH and, GPx of plasma, liver, and brain (P<0.05). The red group resulted the highest in plasma, liver, and brain MDA. The mean plasma MDA values were determined to be 5.573 nmol/g, the mean liver MDA values were 101.800 nmol/g, and the mean brain values were 136.253 nmol/g in the red light group. The second highest group in the plasma MDA was the orange light group and, its mean value was 4.825 nmol/g. For the plasma MDA, the red and yellow groups were statistically different, but purple, blue, and yellow were similar amongst themselves (Table 1). Followed by the red light group, the liver MDA was higher in the white light group with a value of 90.405 nmol/g. Only the blue light group was found to be different from the other groups for the liver MDA (Table 1).

Table 1 Effect of light spectrum on some oxidative stress parameters in plasma, liver and brain tissues of rats

Groups (N=112)		**Plasma MDA**	**Plasma GSH**	**Plasma GPx**	**Liver MDA**	**Liver GSH**	**Liver GPx**	**Brain MDA**	**Brain GSH**	**Brain GPx**
Purple		3.606^{c}	117.771^{cd}	43938.3^{ab}	80.434^{a}	83.906^{c}	37699.6^{b}	134.161^{ab}	8.397^{c}	112089.2^{a}
Blue		3.548^{c}	114.164^{d}	53700.0^{a}	43.044^{b}	108.694^{b}	36278.4^{b}	100.865^{bc}	126.034^{a}	113681.4^{a}
Green		4.051^{bc}	123.125^{c}	51159.8^{a}	89.871^{a}	105.669^{b}	46230.0^{ab}	114.798^{abc}	113.582^{b}	62578.8^{c}
Yellow		3.752^{c}	118.470^{cd}	39756.6^{b}	89.648^{a}	117.422^{b}	64494.6^{a}	128.953^{ab}	115.793^{ab}	44791.8^{c}
Orange		4.825^{ab}	129.991^{b}	49010.6^{ab}	82.615^{a}	106.949^{b}	37572.0^{b}	89.337^{b}	111.836^{b}	103002.1^{ab}
Red		5.573^{a}	132.551^{b}	43188.9^{ab}	101.800^{a}	111.022^{b}	36149.0^{b}	136.253^{a}	112.651^{b}	75893.6^{bc}
White		4.376^{bc}	150.356^{a}	42928.6^{ab}	90.405^{a}	140.581^{a}	25718.9^{b}	129.665^{ab}	126.732^{a}	76267.9^{bc}
P		*<0.001*	*<0.001*	*0.035*	*<0.001*	*<0.001*	*<0.001*	*0.001*	*0.004*	*<0.001*
Male		4.233	124.688	44581.45	77.986	110.124	46144.3	123.070	110.989	76652.5
Female		4.262	128.578	47899.35	87.105	111.088	35039.3	115.223	115.876	91434.6
P		*0.862*	*<0.001*	*0.177*	*0.027*	*0.665*	*<0.001*	*0.214*	*0.129*	*0.001*
Male	Purple	3.570	118.935	38339.4^{ab}	80.479	86.117	31275.5^{c}	137.187	88.910^{b}	112672.9^{a}
	Blue	3.062	111.487	60610.6^{a}	34.898	111.953	41115.7^{c}	110.925	119.400^{ab}	117666.6^{a}
	Green	3.935	120.565	47201.1^{ab}	95.079	99.850	63279.3^{b}	108.076	113.814^{ab}	28280.5^{c}
	Yellow	3.926	117.073	31785.2^{b}	87.690	114.280	89050.6^{a}	139.146	109.159^{ab}	30188.1^{c}
	Orange	5.217	128.478	51970.9^{ab}	43.622	106.367	36462.4^{c}	88.045	106.832^{ab}	103826.8^{a}
	Red	5.769	130.806	41769.7^{ab}	113.062	110.091	36070.4^{c}	142.796	114.280^{ab}	63682.8^{b}
	White	4.149	145.469	40393.3^{ab}	91.073	142.210	25756.2^{c}	135.318	124.521^{a}	80249.8^{b}
Female	Purple	3.641	116.608	49537.2^{A}	80.390	81.695	44123.8^{A}	131.133	85.884^{D}	111505.5^{A}
	Blue	4.033	116.840	46789.5^{AB}	51.189	105.436	31441.1^{AB}	90.805	132.667^{A}	109696.2^{A}
	Green	4.166	125.685	55118.6^{A}	84.663	111.487	29180.7^{AB}	121.519	113.349^{C}	96877.2^{AB}
	Yellow	3.579	119.866	47728.0^{AB}	91.607	120.565	39938.7^{AB}	118.759	122.426^{ABC}	59395.6^{C}
	Orange	4.433	131.504	46050.2^{AB}	121.608	107.530	38681.6^{AB}	90.627	116.840^{BC}	102177.3^{A}

	Red	5.377	134.297	44608.2^{B}	90.538	111.953	36227.7^{AB}	129.709	111.021^{C}	88104.5^{AB}
	White	4.603	155.244	45463.8^{AB}	89.737	138.952	25681.6^{B}	124.011	128.943^{AB}	72286.0^{BC}
P		*0.126*	*0.083*	*0.034*	*0.209*	*0.328*	*<0.001*	*0.757*	*0.038*	*<0.001*
Pooled SEM		0.124	1.642	1405.69	3.607	2.349	2435.54	3.655	1.889	4260.6

$^{a, b, c, d}$: The difference between the means with different letters in the same column is significant (P≤0.05). ^{a,b,c}: Shows the differences of male rats in terms of the averages examined in different color groups. A, B, C: Shows the differences of female rats in terms of the averages examined in different color groups. MDA: Malondialdehyde (nmol/g), GSH: Glutathione (µmol/g), GPx: Glutathione peroxidase (IU/g prot, dry weight)

Brain MDA of the purple light group was measured as 134.161 nmol/g after the red light group. The red and orange light groups were different in point of brain MDA. The plasma GSH resulted in the highest value with 150.356 µmol/g in the white light group. The red and orange light groups among themselves; the yellow, green, blue, and purple were similar among themselves for the plasma GSH level. As in plasma, the highest GSH level in the liver (140.581 µmol/g) and brain (126.732 µmol/g) were in the white light group (Table 1). The mean plasma GSH levels were 132.551, 129.991, 123.125, 118.470, 117.771, and 114.164 µmol/g in red, orange, green, yellow, purple, and blue, respectively. The red and orange light groups were similar. The GSH levels in liver were 117.422, 111.022, 108.694, 106,949, 105.669, and 83.906 µmol/g in yellow, red, blue, orange, green, and purple. The blue, green, yellow, orange, red light groups were similar among themselves. The purple, white and other light groups were different in terms of liver GSH levels (Table 1). The brain GSH level, the purple light group was observed the lowest with a value of 8.397 µmol/g. The blue, yellow, and white light groups appeared as similar groups for the brain GSH levels. Similar to plasma GSH levels, the red and orange groups were determined to be similar in brain tissue GSH levels. In the white, blue, yellow, green, red, and orange light groups brain GSH levels were measured as 126.732, 126.034, 115.793, 113.582, 112.651, 111.836 µmol/g, respectively. The plasma GPx activity was determined as 53700.0 IU/g prot in the blue light group as the highest. Then, green, purple, orange, red, white and yellow light groups were sorted with 51159.8, 49010.6, 43938.3, 43188.9, 42928.6, 39756.6 IU/g prot values. The activity of GPx of the liver tissues were higher in the yellow (64494.6 IU/g prot) and green (46230.0 IU/g prot) light groups than in the other groups (Purple: 37699.6, Orange: 37572.0, Blue: 36278.4, Red: 36149.0, White: 25718.9 IU/g prot). Although the yellow and green light groups were similar the other light groups were different from those groups. However, the yellow light group was also similar to the other light groups (Table 1). In contrast, the brain GPx enzyme activity was observed to be lower in green (62578.8 IU/g prot) and yellow (44791.8 IU/g prot) groups compared to other groups (Blue: 113681.4, Purple: 112089.2, Orange: 103002.1, White: 76267.9, Red: 75893.6 IU/g prot). The brain GPx enzyme activity of the blue and purple light groups were similar (Table 1). In this study, some of the examined parameters were different for the gender basis. The plasma GSH, the liver MDA, the liver and brain GPx were different in male and female rats (P<0.05). Only the liver GPx enzyme activity was higher in male rats, the other parameters in the female rats had a higher value. The plasma GSH was 124.688 µmol/g in male rats, the 128.578 µmol/g in female rats. The liver MDA was 77.986 nmol/g in male rats, the 87.105 nmol/g in female rats. The liver and brain GPx in male rats were 46144.3 and 76652.5 IU/g prot; in female rats were 35039.3 and 91434.6 IU/g prot, respectively. In addition, there were some interactions were observed between gender and light groups regarding the plasma, liver, and brain GPx, and in the brain GSH levels (P<0.05).

4. Discussion

Oxidative stress emerges with the effect of many factors. Today, many studies have been conducted to determine the factors that cause the formation of oxidative stress. It is accepted that the presence of oxidative stress in the formation of many diseases cannot be ignored. In living organisms, reactive oxygen species are produced as a result of normal cellular metabolism and environmental factors. These molecules are highly reactive and damage the other essential molecules such as proteins and DNA and affect cell, tissue, and organ function. However, the harmful effects of the reactive oxygen species are scavenged by the antioxidants. If this scavenging function is insufficient or oxidants are in excessive amounts, an imbalance occurs and this is defined as oxidative stress [10,11]. There are two main defense systems against oxidative stress: the antioxidant enzyme system and low molecular weight antioxidants. One of the best-known antioxidant enzymes is GPx. This enzyme is a cytosolic enzyme that catalyzes the reduction of hydrogen peroxide to water and oxygen as well as the reduction of peroxide radicals to alcohol and oxygen [12]. Another antioxidant that is a member of low molecular weight antioxidants is GSH. GSH is a tripeptide and has multiple functions in organisms. Its antioxidant function is to interact directly with reactive oxygen and nitrogen species [13]. MDA level is a marker of oxidative stress and one of the cells' final products of polyunsaturated fatty acids peroxidation [14]. The eye is vulnerable to oxidative stress due to several numbers of factors. The membrane of the photoreceptor disc of the retina riches in polyunsaturated fatty acids. Another factor is the retinal pigment epithelium produces a high amount of reactive oxygen species because of the extreme oxygen consumption [15]. In addition, some molecules (melanopsins) in the eye capture and detect the intensity of light and affect to other body parts [16]. Similar to the eye, the brain riches

in polyunsaturated fatty acids, and the high energy demand of this organ. Thus, the brain is also susceptible to oxidative stress [17]. Light is necessary for organisms to regulate their normal physiological conditions. On the other hand, many creatures interact with light naturally or artificially in their environment. Unsuitable artificial light conditions may become an abiotic stress factor in living organisms. These unsuitable conditions are related to irregularity by light intensity, wavelength, and type of light source (traditional or light-emitting diodes) [1,18]. To date, the detrimental effects of blue light have been proven in human [19,20]; however, there are few studies have been conducted on the detrimental effects of other light spectrums. In this study, the fluorescent blue light has lower MDA in the plasma, liver, and brain of the rats than the other light colors. In contrast, red light has superior MDA levels in the plasma, liver and brain of the rats. In another study that was similar to our results, the red light induced oxidative stress in yellowtail clownfish as well as in chickens [21,22]. This fact is associated with red light induces lipid peroxidation in polyunsaturated fatty acid riched tissues. In addition, this difference is sourced by different reactions to colors by different species due to cellular and tissue differences in the eye. In a study that compares different light spectrum actions on oxidative stress [23], an increase in MDA also increases GSH levels which is consistent with our results. The blue light also increased the plasma and brain GPx activity in this study. The short wavelength light (~380-445 nm) such as blue and purple is detected as phototoxic by mitochondria and activates the antioxidant mechanism [23]. Although it is not rich in polyunsaturated fatty acids, unlike those in the brain and eyes, the liver has high metabolic activity, and this makes the liver susceptible to oxidative stress [24]. Every organ may have a different defense mechanism by activating different molecules against oxidative stress, but the basic mechanism is the same: to activate scavengers for oxidative stress-leading agents. Therefore, the antioxidant enzyme activities or levels of the antioxidant agents may differ in different organs. Indeed, in this study, the levels of GSH and GPx were different in plasma, liver, and brain. In this study, there were gender differences observed in oxidative stress. These differences were observed in plasma GSH, liver MDA, liver, and brain GPx. Some studies show females have lower oxidative stress levels and reactive oxygen species production than males. The main reason for gender differences is sourced by estrogen acts as an antioxidant by scavenging free radicals due to the presence of a phenolic hydroxyl group. In particular, the GPx is less required in females [25]. In a previous study, it was shown that white light has more negative effects on females than males. On the other hand, yellow light had no negative effect on males in contrast to white light and unlike females [16]. In this study, there were significant interactions determined between gender and light color. The white light caused more plasma MDA and GPx in female rats. The plasma, brain GPx and brain GSH levels were low in male rats.

5. Conclusion

In conclusion, low-wavelength lights may be more useful for rats' environments when compared to high wavelengths in manner oxidative stress. However, illumination is not the sole phenomenon for raising animals. Environmental demands may vary from time to time and even according to gender. Moreover, it may be useful for studies to be conducted by considering other lighting factors such as intensity and cycle.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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