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Original Article

Fluorescent Light Induced Oxidative Damage in Wistar Strain Albino Rat : Possible Protective Effect of LED Light Induced Photobiomodulation

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Abstract

Fluorescent light exposure affects normal physiology in both animal models and human. Primary objective of this study was to elucidate the effects of 1800 lux fluorescent light exposure on oxidative stress markers. Additionally, effects of 670 nm LED light exposure on fluorescent light induced changes were also elucidated. Wistar albino rats were divided into 10 groups based on fluorescent / LED light exposure for 1, 15 or 30 days group. Oxidative stress markers like lipid peroxidation, total reduced glutathione and super oxide dismutase in brain, heart, kidney, liver, skeletal muscle and blood were analysed. One-way ANOVA and Tukey's multiple comparison tests were used for statistical analysis. Exposure to fluorescent light resulted in oxidative damage and LED light offered protection against this oxidative damage. Protective effect of LED light against fluorescent light induced damage might be due to photooxidation and enhanced antioxidant activity.

Introduction

Fluorescent lighting has become an unavoidable source of light in both day and night time, making human to adapt to 24 hour active society. However, increase in the use of fluorescent lighting during night time produce undesirable side effects known as "light pollution". Light pollution cascade numerous physiological changes in a living organism at the cellular level. Even minor deviation in the intensity and duration of fluorescent light at a given time of day/night can alter or disrupt physiology. Various studies on animal models have suggested that use of fluorescent light especially during night time results in multisystem deleterious and harmful effects. Cardiovascular, neuro muscular, hepato renal and even metabolic and endocrinological disturbances have been reported (1-15). Hence, deleterious effect of light exposure at night has to be taken into consideration.

Oxidative stress is implicated in the development of

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various pathological states in humans as well as experimental animals. Alteration in circadian rhythm and melatonin levels is implicated in the oxidative damage due to artificial fluorescent light exposure. The cellular damage is reflected by the change in concentrations of Lipid peroxidation (LPO), glutathione (GSH) and superoxide dismutase (SOD). The present study aims at finding the possible link between tissue damage and oxidative stress which results due to light pollution (16). However, there are conflicting reports on the levels of antioxidants and their byproducts in the available literature. Constant light exposure at night in rat for a period of 12 hours upto 21 days increases liver, kidney as well as circulatory thiobarbituric acid reactive substance (TBARS) and decreases antioxidative enzymes GSH and SOD (17). Light exposure of 500 lux (lx) between 9.00 am to 1.00 pm for a period of 7 days to rats increases brain TBARS and decreases GSH and SOD (18). In contrast, mice exposed to 30 min light for 2, 7 and 21 days showed gradual increase in liver SOD activity with increase in the acclimation period (4). Another study shows that continuous light exposure increases GSH and SOD level in rat brain (19). Studies have also explored the therapeutic potentials of fluorescent light exposure (20, 21). Based on the available literatures, it is clear that light exposure causes imbalance in oxidative process, though some are conflicting with tissue specific variations in antioxidant defense mechanism in different tissues. However, tissue specific interactions as well as time dependent modification in light induced oxidative stress have not been documented extensively. Hence, we investigated the effect of fluorescent light exposure on three key oxidative stress markers LPO, GSH and SOD.

Melatonin supplements are being commonly used as treatment to overcome light induced damages (22). However, it is expensive and time consuming and so there is a need for an alternative. One such alternative is LED (Light Emitting Diode) light exposure, because it is known to have antioxidant activity. Hence, utility of LED exposure in fluorescent light induced biochemical changes were also evaluated in this study.

Methods

Toxic effects of light at night in animal model mimic similar effects on humans (23, 24). Rat is a suitable model to study the effects of light induced stress (25). Hence, Wistar strain albino rats have been chosen for the study. On obtaining the ethical clearance from Institutional animal ethics committee, male Wistar rats weighing between 150-170 gms were divided into 10 groups (n=6 each). The animals were divided based on their exposure as described below.

Group 1 [CL] was control animals maintained at normal ambient animal house temperature, illumination and light cycle (12L: 12 D). Group 2, 3, 4 were one day exposure groups (Fluorescent light exposure of 1800 lx [FL_1], LED light exposure + fluorescent light exposure of 1800 lx [LL_1] and only LED light exposure [OL_1]). Similarly the animals were grouped based on the exposure regimen and days of exposure into fifteen day groups - Group 5[FL_{15}], 6[LL_{15}], 7 [OL_{15}] and thirty day groups - Group 8[FL_{30}], 9[LL_{30}] and 10[OL30]. The experimental set up and methods utilized for fluorescent light exposure of 1800 lx and LED light of 670 (±10) nm in the near infra red range with energy density of 9J/ cm^2 are as described in detail in our earlier publication (26). Fluorescent light exposure was between 8 p.m – 8 a.m daily for 1, 15 or 30 days. LED light of 670 nm for duration of 6 min was exposed independently or prior to fluorescent light exposure as per the group criteria.

By using pentothal sodium as a mode of anaesthesia, animals belonging to each group were euthanized and blood, brain (cerebral cortex), heart (cardiac muscle), liver, kidney and thigh muscle (skeletal muscle) of the animals were harvested for LPO (27), GSH (28) and SOD (29) estimation. Rats belonging to 1 day exposure group were sacrificed immediately after the exposure, however rats belongs to 15 and 30 day groups were sacrificed 24 hours after their final exposure to avoid acute exposure effects on the parameters studied. After the harvest of the organs and tissues, the carcasses were disposed as per the CPCSEA guidelines.

All statistical computations were performed using SPSS statistical package (Version 17.0). Values of each group are given as graphical representation. One-way ANOVA and Tukey's multiple comparison tests were used to determine statistical significance. $P<0.05$ was considered statistically significant.

Results

Control vs fluorescent light

Tissue and plasma lipid peroxidation showed a significant rise after exposure to fluorescent light for a period of 15 (FL_{15}) and 30 (FL_{30}) days (Table I). This was accompanied with a fall in total reduced glutathione. Reduction in the levels of reduced glutathione however was observed after exposure to one day of fluorescent light (FL_1) itself, except in the skeletal muscle where this change was observed only after 15 and 30 days of exposure (Table II). The antioxidant enzyme, superoxide dismutase level was significantly lowered in all the three points (FL_1, FL_{15} & FL_{30}) studied in the liver. This fall however was observed only after 15 days of exposure in the brain, muscle and hemolysate. No significant change were observed in the heart and kidney (Table III).

Control vs LED pre exposure

LED pre exposure groups (LL_1, LL_{15} and LL_{30}) showed significant change in the parameters studied when compared with the control group. Lipid peroxidation was higher in skeletal muscle at all the three time points studied. Similar higher values were observed in the brain and liver after exposure for 15 and 30 days and in the kidney of the 30 day group alone. Liver is the only organ which showed a significant lower value for lipid peroxidation after 30 days of LED pre exposure (Table I). Total reduced glutathione showed significant decrease in the LL_1 group in all the tissues except skeletal muscle. A rise was observed after 15 and 30 day LED pre exposure (LL_{15}, LL_{30}) in the tissues studied. Liver was one of the tissues studied where lower levels of total reduced glutathione was observed both in 1 and 15 day exposure and the rise observed only after 30 days exposure (Table II). A similar change was observed with Superoxide dismutase. Though the levels of SOD after one day exposure (LL_1) showed a decrease in the brain, heart and kidney they were statistically not significant (Table III).

Control vs only LED exposure

A significant change in the parameters studied was observed in all the tissues after exposure to only LED light for a period of 30 days (Table: I, II and III). The rise in GSH was significant in the plasma, brain, heart and kidney, with a concomitant fall in LPO observed only in the brain and plasma in the OL_{30}

TABLE I: Lipid Peroxidation (µmols of MDA/ml of plasma or homogenate).

	Days	*CL*	*FL*	*LL*	*OL*
Plasma	1 day	7.482±0.574	6.826±0.154	6.796±0.338	7.774±0.551
	15 days	7.482±0.574	16.599±0.226*$	11.975±0.358*#$	7.745±0.225
	30 days	7.482±0.574	13.419±0.832*$@	6.637±0.458#@	5.747±0.199*$@
Brain	1 day	6.870±0.174	7.132±0.267	6.972±0.754	7.060±0.483
	15 days	6.870±0.174	15.257±0.440*$	8.226±0.749*#$	6.111±0.299$
	30 days	6.870±0.174	12.398±0.175*$@	11.391±0.335*#$@	6.155±0.181*$
Heart	1 day	6.520±0.271	6.214±0.139	6.330±0.417	6.666±0.129
	15 days	6.520±0.271	14.498±0.443*$	7.541±0.279*#$	6.593±0.404
	30 days	6.520±0.271	14.571±0.525*$	9.422±0.514*#$@	6.399±0.174
Kidney	1 day	7.293±0.415	8.926±0.248*	7.060±0.208#	6.884±0.126
	15 days	7.293±0.415	8.795±0.161*	6.826±0.404#	6.651±0.126
	30 days	7.293±0.415	14.119±0.434*$@	11.348±0.259*#$@	6.680±0.259
Liver	1 day	9.408±0.210	9.393±0.320	8.926±0.943	8.999±0.153
	15 days	9.408±0.210	14.484±0.388*$	8.955±0.215#	8.941±0.235
	30 days	9.408±0.210	15.009±0.732*$	6.432±0.153*#$@	9.072±0.615
Skeletal Muscle	1 day	3.894±0.204	5.951±0.196*	6.301±0.309*	3.326±0.360
	15 days	3.894±0.204	8.970±0.494*$	5.411±0.331*#$	3.296±0.757
	30 days	3.894±0.204	9.145±0.876*$	7.176±0.469*#$@	3.325±0.355

TABLE II : Reduced Glutathione (µg/ml of hemolysate or homogenate).

	Days	CL	FL	LL	OL
Hemolysate	1 day	19.092±0.413	14.502±1.631*	16.227±1.855*	18.669±0.800
	15 days	19.092±0.413	9.359±1.197*$	22.689±2.281*#$	19.238±0.347
	30 days	19.092±0.413	15.406±0.591*$	25.629±0.418*#$	23.293±0.922*$@
Brain	1 day	11.865±0.262	9.229±1.235*	9.131±1.060*	11.507±0.618
	15 days	11.865±0.262	7.048±0.354*$	16.683±1.072*#$	11.605±0.246
	30 days	11.865±0.262	10.726±0.450*@	23.177±0.119*#$@	13.346±0.138*$@
Heart	1 day	36.833±1.334	17.448±2.055*	17.301±2.317*	36.182±1.199
	15 days	36.833±1.334	27.588±1.044*$	50.049±1.610*#$	36.751±1.454
	30 days	36.833±1.334	26.812±1.665*$	59.629±0.565*#$@	40.333±2.096*$@
Kidney	1 day	37.646±1.528	23.112±3.027*	22.314±4.224*	36.230±1.452
	15 days	37.646±1.528	28.955±2.665*$	47.868±2.014*#$	36.426±1.171
	30 days	37.646±1.528	11.545±0.206*$@	40.199±0.323#$@	41.814±2.768*$@
Liver	1 day	50.684±1.517	21.322±3.173*	21.094±3.221*	48.739±0.871
	15 days	50.684±1.517	29.248±0.669*$	33.919±2.645*#$	49.007±2.429
	30 days	50.684±1.517	25.379±0.415*$@	54.001±1.365*#$@	50.102±0.685
Skeletal Muscle	1 day	29.948±1.033	29.370±0.423	27.976±1.869	29.097±0.514
	15 days	29.948±1.033	26.774±0.354*$	32.861±0.329#$	29.346±2.533
	30 days	29.948±1.033	21.425±0.412*$@	33.160±0.282*#$	29.187±0.259

TABLE III : Superoxide Dismutase (50% inhibition of pyrogallol auto oxidation/min/ml of hemolysate or homogenate).

	Days	CL	FL	LL	OL
Hemolysate	1 day	27.037±1.418	14.074±1.913*	15.926±3.285*	28.148±1.210
	15 days	27.037±1.418	21.481±0.855*$	42.963±1.711*#$	31.111±1.711*
	30 days	27.037±1.418	17.574±1.211*$@	52.482±3.289*#$@	32.259±4.271*
Brain	1 day	26.296±2.222	20.370±2.530	20.741±6.735	26.667±1.210
	15 days	26.296±2.222	24.444±0.855$	33.704±2.222#$	29.259±2.804
	30 days	26.296±2.222	17.100±1.510*@	41.100±4.530*#$	39.900±5.745*$@
Heart	1 day	22.347±1.235	17.037±1.913	18.148±5.042	26.667±2.095
	15 days	22.347±1.235	22.222±1.711$	28.148±3.421$	37.778±0.855*$
	30 days	22.347±1.235	18.670±2.630	53.907±2.332*#$@	46.019±3.021*$@
Kidney	1 day	25.593±1.298	26.167±0.333	27.241±2.650	28.815±1.532
	15 days	25.593±1.298	21.481±0.855$	40.000±5.132#	28.889±0.855
	30 days	25.593±1.298	21.519±2.927$	35.044±4.318#	39.963±1.587*$@
Liver	1 day	36.898±3.067	22.593±3.285*	21.852±6.667*	32.593±3.825
	15 days	36.898±3.067	18.519±0.855*	22.963±2.566*	34.074±3.421
	30 days	36.898±3.067	23.822±2.133*@	46.578±5.369*#$@	43.022±3.740$@
Skeletal muscle	1 day	26.259±2.182	20.370±5.324	21.852±5.722	26.296±4.895
	15 days	26.259±2.182	18.519±0.855*	41.111±1.418*#$	27.407±1.913
	30 days	26.259±2.182	20.278±1.078*	44.157±1.950*#$	42.226±2.979*$@

Values given are Mean±SD. Each group consisted of 6 animals.
Groups: CL - control, FL - fluorescent light exposed group, LL - LED pre exposure + fluorescent light exposed group, OL - only LED exposed group
ANOVA was performed followed by Tukey's multiple comparison if F test ratio was significant.
Level of significance was set at $p<0.05$
Comparison between groups denoted by superscripts:
*denotes significant difference with CL
#denotes significant difference between FL and LL
Effect of Time denoted by superscripts:
$denotes comparison with their respective 1 day group
@denotes comparison between their respective 15 and 30 days group

group. Levels of SOD showed marked rise in the plasma and heart after exposure to only LED light for 15 and 30 days. This rise was observed in other tissues only after 30 days of exposure.

Fluorescent light vs fluorescent light + LED pre exposure

Pre exposure of animals to LED light before exposing them to fluorescent light showed a significant lower level of lipid peroxidation in all the organs studied in the LL_{15} and LL_{30} groups of animals (Table I). In these tissues similar change was observed in the levels of SOD and GSH following pre exposure to LED light (Table II & III). In the plasma, LPO showed no change in the LL_1 and LL_{30} group, however a significant rise was observed in the LL_{15} group alone. Superoxide dismutase and total reduced glutathione levels showed significant rise similar to the changes observed in the tissues studied in the LL_{15} and LL_{30} group.

Discussion

Radiation exposure from manmade sources as simple as X ray and television affects human physiology, metabolism and behavior. The degree of damage by the various radiation exposures depends upon the source, time as well as duration of exposure. In the same way, artificial light exposure has the ability to disrupt the physiological mechanism of living organisms. Fluorescent light exposure during the night causes more damage than exposure during the day (30). In our study, lipid peroxidation, peroxidation of polyunsaturated fatty acids, GSH - natural antioxidant and SOD - first line defense enzyme against reactive oxygen species (ROS) in the cell were assayed to quantify the degree of oxidative damage. The levels of these markers were altered in the blood as well as in other tissues following exposure to fluorescent light, which was duration and tissue specific.

Light exposure at night can result in oxidative stress in a duration and intensity dependent manner. One of the important causes for the oxidative stress is decreased levels of the antioxidant hormone, melatonin which is altered with light exposure at night as seen in our earlier study (31). From the present study, it is evident that exposure of rats to fluorescent light at night results in increased lipid peroxidation in the tissues examined right from day one of exposure. This result is in agreement with earlier studies using light of other lower lx. Even exposure of rats to constant light in a standard animal house lighting (325 lx) results in increased lipid peroxidation in the brain, liver and kidney when exposed to 2 weeks (32) and in the blood following a period of 21 days (17). Hence, it is clear from the results obtained in our study that fluorescent light exposure induces oxidative damage and is dependent on the duration of exposure and is also tissue specific (26, 31).

Mechanisms for fluorescent light induced oxidative damage have been documented in earlier studies. Increase in lipid peroxidation is probably due to the generation of photo-oxidants and reactive radicals (32), stimulation of flavins, an endogenous photosensitizer, to initiate generation of free radical (33). Photosensitized reaction can occur by means of type I and II reactions, where it leads to the production of ROS (34). Photo oxidative reaction resulting in increased lipid peroxidation of brain tissue following fluorescent light exposure has been studied and explained with glutamate N methyl – D aspartate (NMDA) receptor and calcium ion concentration. These finally result in the production of toxic hydroxyl radicals (17).

Change in glutathione system was time dependent and the first to occur with a decrease in its concentration seen in almost all the tissues. This could be due to over utilization of GSH to scavenge the fluorescent light mediated lipid oxidation. Other possible reason for decrease in GSH might be due to the low melatonin concentration (35) or direct inhibition of glutathione peroxidase activity by the fluorescent light (36). Decrease in SOD in the FL group of animals in the various tissues and time points indicates over utilization of this catalytic enzyme to quench the free radicals generated by fluorescent light exposure. Earlier reports following exposure to fluorescent light of varied lx have also observed similar changes as observed in our study with respect to GSH and SOD levels in the blood

and other tissues (17, 32). Few of the tissues studied did not show any significant change. This can be attributed to variation in the dominant antioxidants in these tissues or quantum of light required to produce oxidative damage could be tissue specific.

Exposure to 30 days of LED increased the total reduced glutathione and SOD level significantly. Protective effect of LED on oxidative stress and oxidative damage has been evaluated extensively. Exposure to LED of 670 nm with an intensity 9 J/cm^2, as used in the current study, for 18 days as well as 14 weeks has shown partial protection in diabetic rats as observed in the liver antioxidant status and damage (37). Similarly another animal study with the same wavelength of LED exposure however with intensity of 6 J/cm^2 also exhibited similar protective response by inhibiting free radical induced early lesions in diabetic retinopathy (38). In the current study, this protective effect of LED was evident in the LED pre exposure followed by exposure to fluorescent light as well. LL_{15} group animals showed significant change in the oxidative stress parameters, when compared to FL_{15} group and their values were near control values. Most of these changes returned to baseline or control value in the LL_{30} group. This indicates that that LED pre exposure offers a significant protection in all the tissues studied from free radical induced oxidative damage effect of fluorescent light. As the changes observed in the LL_1 group were identical with FL_1 group it indicates that LED light protective effects depend on duration of exposure (26).

The LED protective effect might be due to stimulating effect of the redox signaling in cells, enhanced antioxidant activity and/or direct effect on reducing free radical damage (37, 39, 40). Evidences from the previous literature show that low power laser irradiation in the red to near infrared region enhances cellular metabolic functional activity and reducing lipid peroxidation in cell (41). LED light exposure in the near infrared region might also exhibit the protective response via a similar mechanism. In our earlier study, we found that LED light exposure of 670 nm for period of thirty days showed significant protection against 1800 lx fluorescent light induced retinal damage. This was evident with increase in outer nuclear cell count and thickness (26, 31). Light exposure in the near infra red (NIR) wavelength act as a therapeutic/optical window in biological tissues, as this wavelength of light has a better and deeper light tissue interactions (42).

Chromophores are responsible for photobiostimulation. Endogenous porphyrins, mitochondrial membrane cytochromes and flavoproteins act as photo acceptors in visible light and NIR light region (43). Among this, the key role is played by cytochrome c oxidase, as the absorption spectra of cytochrome c oxidase and action spectra of low intensity red and NIR light are the same for any biological response (42). However, one should take into account that cytochrome c oxidase act as a primary photo acceptor only in partially reduced form. In addition, red and near infrared light exhibit a biphasic response, i.e., induces as well as decreases ROS production depending upon the intensity and duration of exposure (44). Hence, a short duration of LED pre exposure, as used in our study (26, 31), might result in activation of cytochrome c oxidase, alters redox status, reduces the oxidative stress and radical damage (37). It could also enhance transcription factor activity or production and increase energy metabolism (41). In this study we have seen that LED light photobiomodulation is contributed by the biochemical and cellular changes at macroscopic level, like changes in oxidation and reduction reactions of glutathione system and antioxidative enzymes like SOD.

Conclusion

Fluorescent light exposure results in oxidative stress. LED light therapy improves the antioxidant defense system and protects the tissues from oxidative damage thereby indicating that 670 nm LED light photobiomodulation may be broadly applicable to reverse fluorescent light induced oxidative stress. The current study was done for specified period of exposure to fluorescent light/LED light exposure which is a limitation of our study. Additional studies are necessary to elucidate the exact mechanism(s) of this protective effect.

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