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Redox homeostasis in a rodent model of circadian disruption: Effect of melatonin supplementation

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ABSTRACT

Continuous light or dark photoperiods are the leading cause of disruption in the circadian rhythm of day-night cycle. The purpose of this study was to understand the cellular redox balance in a model of circadian disrupted rat model and determine the effect of melatonin supplementation. Young male Wistar rats were randomly divided into five groups (n = 4). Group (I): normal day-night (12 h:12 h) cycle, Group (II): normal rats treated with melatonin, Group (III): rats subjected to continuous light exposure (CLE), Group (IV): CLE rats treated with melatonin, and Group (V) Rats subjected to continuous dark. Melatonin (10 mg/kg) was administered orally at dusk to the Group (II) & (IV). Rats were sacrificed after 10 days of treatment and biomarkers of oxidative stress were evaluated. Results demonstrated significant ($p < 0.05$) increase of malondialdehyde (MDA), plasma membrane redox system (PMRS), protein carbonyl oxidation (PCO), advanced oxidation protein products (AOPPs), and advanced glycation end products (AGEs) during CLE. A significantly ($p < 0.05$) decreased level of reduced glutathione (GSH) and ferric reducing antioxidant potential in plasma (FRAP) was also observed during CLE. Treatment with melatonin in CLE rats showed reduced level of MDA, PMRS, PCO, AOPPs and AGEs while GSH and FRAP activity were increased. During continuous dark exposure (CDE) the biomarkers of oxidative stress were attenuated compared to control. Supplementation of melatonin could be a promising strategy to maintain redox homeostasis during prolonged condition of light exposure and other conditions of redox imbalance.

1. Introduction

Circadian rhythm helps organisms to synchronize their behavior and physiology with the daily alteration of light and dark phases (Schmal et al., 2015). The suprachiasmatic nucleus (SCN) located in the hypothalamus is recognized as the primary pacemaker coordinating the diverse rhythmicity of various circadian rhythms and aiding in the synchronization of peripheral oscillations (Albrecht, 2012). The SCN activity is governed by the photic information (light/dark) perceived through eyes and further redirected to the pineal gland, coinciding with the production of the pineal hormone, melatonin, in the dark phase. Melatonin (*N*-acetyl-5-methoxytraptamine), not only regulates circadian rhythms and sleep via its specific receptors, but also reportedly exhibits a direct free radical scavenger activity and acts as a potential antioxidant (Han et al., 2017; Johns and Platts, 2014).

It is hypothesized that evolution of melatonin dates back to the period when anaerobic organisms transitioned to aerobic organisms. The evidence from its earliest origin proves that melatonin serves as an antioxidant in bacteria, unicellular organisms and green algae. It is also present in plants, fungi, insects, and vertebrates including mammals (Tan et al., 2013). The unique broad spectrum antioxidant property of melatonin makes it a very basic substance present in all living organisms (Tan et al., 2010). This assumption is based on melatonin's ability to organize the circadian biology of cells and also to its action as a direct free radical scavenger coupled to its indirect actions in the promotion of enzymatic antioxidative defense process (Venegas et al., 2012).

In mammals including human, pineal melatonin synthesis is strictly restricted to the dark phase of the light/dark cycle (Tan et al., 2014). During the day hours, light blunts the norepinephrine release to the pinealocyte, inhibiting AANAT (Arylalkylamine *N*-acetyltransferase), a rate limiting enzyme in melatonin synthesis and melatonin production. In rats, nocturnal restricted upregulation of AANAT mRNA is contributed to the 150-fold increase in the pineal melatonin synthesis during night as compared to the daytime (Schwartz et al., 2009). A condition of continuous dark exposure (CDE) contributes to a rhythm free-running which dramatically increase pineal melatonin synthesis, preserves the internal synchrony because of intact melatonin and SCN

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output signals. However these changes can be amended by manifestation of external signals including light (De Magalhaes Filho et al., 2018).

Light is a robust constituent in circadian, neuroendocrine and neurobehavioral ordinance and it has an overwhelming impact on health and welfare of all mammals including laboratory animals (Hardeland, 2008; Nakamura et al., 2008). Exposure of light greater than 100 lux has been reported to cause suppression in the serum melatonin level and its pineal biosynthesis (Arendt, 1998). Human exposure to excess artificial light has been reported to provoke ROS production throughout the body (Chang et al., 2015) and cause oxidative damage to the cellular elements in its target organ (Shin et al., 2011). Continuous light exposure (CLE) of LED spectra can have adverse effects on redox homeostasis including oxidative stress, resulting in the massive increase in the lipid peroxidation (LPO) and oxidative damage to the proteins (Tanito et al., 2006).

With the increasing use of artificial light and the blurring of day/night change in light, it is important to study the role of light as a contributing factor to the redox imbalance. Any study involving redox alteration due to circadian disruption of the day night cycle should be interpreted in terms of the diurnal interruption in the synthesis of melatonin hormone. The present study is an attempt to understand the redox alterations and the resultant cellular homeostasis under conditions of prolonged light and/dark. The present study also reports the effect of continuous light/dark and the effect of melatonin supplementation on rats.

2. Materials and methods

2.1. Chemicals and reagents

Melatonin, 2,4-dinitrophenylhydrazine (DNPH), reduced glutathione (GSH), 4,7-Diphenyl-1,10-phenanthroline disulfonic acid sodium salt (DPI), and 5,5-Dithiobis nitro benzoic acid (DTNB) was purchased from Sigma, St. Louis, USA. Cholesterol and HDL detection kits were procured from Erba Biosciences, USA. All other chemicals of analytical grade were available from Merck, Germany and SRL, India. Thermo Fisher (Evolution-201) UV–VIS spectrophotometer has been used for spectrophotometric measurements.

2.2. Animal model and experimental design

Young male Wistar rats having body weight 150 ± 20 g were housed in a controlled (temperature 20–25 °C and relative humidity 55 ± 15%) conditions with a 12-h light/12-h dark cycle. All rats were fed with a normal laboratory diet of nutrient-rich pellets containing total energy as fat, protein, and carbohydrates, and had free access to drinking water. 20 adult male Wistar rats were randomly divided into five groups (n = 4) as follows:

Group (I): Control (C) 12:12 h (light and dark cycle).
Group (II): Control (C) 12:12 h (light and dark cycle) and supplemented with melatonin (10 mg/kg of body weight) dissolved in physiological saline for 10 days (Agil et al., 2010).
Group (III): Continuous Light Exposure (CLE) for 24 h for 10 days.
Group (IV): Continuous Light Exposure (CLE) for 24 h and supplemented with melatonin (10 mg/kg of body weight) for 10 days.
Group (V): Continuous Dark Exposure (CDE) for 24 h for 10 days.

Group (III) and (IV) animals were maintained under artificial continuous illumination of LED bulb having light intensity of 500 lux measured by digital lux meter (ACCU PLUS LX-101A). The protocol of the study was approved by the Animal Ethics Committee of University of Allahabad.

2.3. Sampling and biochemical assays

Rats were sacrificed under light anesthesia upon the completion of treatment schedule and the blood collected from each rat in heparinized syringes via cardiac puncture. The collected blood from each experimental group was centrifuged at 800*g* at 4 °C for 10 min. Plasma was isolated and stored at −80 °C until the analysis of biochemical parameters. After removing the buffy coat, the upper 15% section of packed red blood cells (PRBCs) was discarded and the remaining PRBCs were washed thrice with ice-cold phosphate-buffered saline (PBS) and resuspended in PBS containing 0.09% glucose. The serum was also isolated from clotted blood after centrifugation and stored at −80 °C for further analysis.

2.4. Estimation of total cholesterol (TC), high-density lipoprotein (HDL) and advanced glycation end products (AGEs) in serum

Serum total cholesterol (TC) and high-density lipoprotein (HDL) were measured using reagent kits from ERBA diagnostics on an Erba Mannheim Chem-7 analyser. Fluorescent AGEs was measured by spectrofluorimetric method describe by Kalousová et al. (2002). Blood serum was diluted 1:50 with PBS (pH 7.4) and fluorescence intensity was read at the excitation/emission of 350/440 nm respectively using Agilent Cary Eclipse Fluorescence Spectrophotometer. Fluorescence intensity was expressed in arbitrary units (AU)/g protein.

2.5. Determination of intracellular ROS in PRBCs

ROS mediated fluorescence in PRBCs was also validated with fluorescent microscopy following our established protocol using DCFH-DA dye (Singh et al., 2016). The microphotographs were captured by Olympus fluorescent microscope (Model: CX21i-TR-LED) equipped with Magnus UHCCD-USB 21.4 megapixel camera.

Intracellular ROS in PRBCs was determined by fluorescence spectroscopic method using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) dye following our earlier described method (Singh et al., 2016). The results were calculated with respect to the control group and the data are expressed in percent change of the control group that was set at 100%.

2.6. Measurement of biomarkers of oxidative stress in erythrocytes

2.6.1. Estimation of malondialdehyde (MDA)

Erythrocyte MDA, an index of lipid peroxidation, was measured based on the reaction with thiobarbituric acid (Esterbauer and Cheeseman, 1990) with slight modification. PRBCs (0.2 mL) were suspended in 3 mL PBS containing 5.0 mM glucose with pH 7.4. Further, the suspended packed erythrocytes was mixed with 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.67% thiobarbituric acid (TBA), boiled for 20 min at 90–100 °C, and then cooled. Subsequently, the mixture was centrifuged at 1000*g* for 10 min and the absorbance of supernatant was read at 532 nm. The concentration of MDA in erythrocytes was calculated using extinction coefficient ($\varepsilon = 153{,}000$) and is expressed as nmol/mL of packed erythrocytes.

2.6.2. Determination of reduced glutathione (GSH)

The erythrocyte GSH content was measured following the method of Beutler (1984), which is based on the ability of the –SH group to reduce 5,5′-dithiobis, 2-nitrobenzoic acid (DTNB) and form an anionic product of yellow colour which strongly absorbs at 412 nm. The erythrocyte GSH concentration was measured and expressed in terms of mg/ml PRBCs.

2.6.3. Measurement of PMRS activity

Erythrocyte PMRS activity was measured according to our previously described protocol (Rizvi et al., 2006). The method is based on reduction of ferricyanide to ferrocyanide which forms complex with DPI, the absorbance of which was measured at 535 nm. The ferrocyanide content was calculated using extinction coefficient ($\mathcal{E} = 20{,}500\,M^{-1}\,cm^{-1}$) and the results are expressed in μmol ferrocyanide/ml PRBCs/30 min.

2.7. Measurement of oxidative stress biomarkers in blood plasma

2.7.1. The measurement of total antioxidant activity by FRAP

The FRAP assessment was performed by following the established protocol of Benzie and Strain (Benzie and Strain, 1996). The method involves the reduction of ferric ion to ferrous ion by plasma based nonspecific antioxidants. The absorbance was read at 593 nm at the interval of 30 s for 4 min. FRAP values [μmol Fe(II)/L] of the plasma was calculated using the standard calibration curve.

2.7.2. Determination of plasma protein carbonyl

Plasma protein carbonyls were measured according to procedure of Levine et al. (1990). The modified version of this procedure has been described in our recent lab reports (Garg et al., 2017). Carbonyl content was determined by taking the spectra of the supernatant at 370 nm. Each sample was read against the control. The carbonyl content was calculated using an absorption coefficient of $22{,}000\,M^{-1}\,cm^{-1}$ and data were expressed in nmol/mg protein.

2.7.3. Determination of advanced oxidation protein products (AOPP)

Plasma AOPP was measured spectrophotometrically by the method of Witko-Sarsat et al. (1996) with minor changes as described previously (Singh et al., 2016). The optical density of chloramine-T at 340 nm being linear within the range of 0–100 μmol/L was used as calibrator to calculate the value of AOPP and concentration was expressed as μmol of chloramine-T equivalent/liter of plasma (μmol/L).

2.8. Statistical analysis

The data obtained in this study are expressed as the mean ± standard deviation (SD). The intergroup variations was measured by one-way analysis of variance (ANOVA) using the Graph Pad Prism, version 5.0. Statistical comparisons were made with “Bonferroni's Multiple Comparison Test”. The values with $p < 0.05$ are considered to be statistically significant.

3. Results

3.1. Effect of melatonin on body weight and some biochemical indices

Table 1. Shows the mean body weight of each group during the period of 10 days. Compared with control group, continuous light exposed (CLE) rats show a significant ($p < 0.05$) increase (45.33%) in the body weight. However non-significant changes in the body weight of melatonin supplemented rats of Group (II) and continuous dark exposed (CDE) group of rats were observed. When compared to the light exposed rats, melatonin supplementation in Group (IV) reduced (34.25%) body weight significantly ($p < 0.05$). The change in serum levels of total cholesterol, HDL, and advanced glycation end products (AGEs) in all experimental groups are represented in the table.

3.2. ROS production and melatonin

CLE group of rats show significant ($p < 0.05$) increment (102.5%) in the intracellular ROS generation as compared to control rats. A non-significant reduction (10%) has been found in Group (II) melatonin treated rats and non-significant increase (10%) in the continuous dark exposed (CDE) rats as compared to control. A significant ($p < 0.05$) reduction (23.95%) in ROS was measured in melatonin supplemented rats when compared to the CLE group. The fluorescence microscopic image analysis (Fig. 1A) for intracellular ROS generation also shows a trend concurring with spectrofluorimetric results (Fig. 1B).

3.3. Effect of melatonin on lipid peroxidation

Fig. 2(A) depicts the malondialdehyde (MDA) values as an index of lipid peroxidation. Significant ($p < 0.05$) progressive increase (41.83%) in erythrocytes MDA level was noted with the continuous light exposed group while significant ($p < 0.05$) decrease (34.59%) has been found to the dark exposed rats as compared to the control group. There was a non-significant reduction (17.30%) in Group (II) melatonin treated rats as compared to control. The formation of MDA in melatonin treated group (IV) decreases (35.32%) significantly ($p < 0.05$) when compared to the CLE group.

3.4. Reduced GSH content after melatonin supplementation

A significant ($p < 0.05$) decline (42.35%) in erythrocytes GSH level was observed in continuous light exposed rats while significant ($p < 0.05$) increase (29.41%) was reported in the continuous dark exposed rats as compared to the control group. While non-significant decrease (20%) in the GSH level has been found in Group (II) melatonin treated rats as compared to control. Corresponding to the light treated group there was a significant ($p < 0.05$) increase (83.67%) in the GSH level in Group (IV) melatonin treated rats Fig. 2(B).

3.5. Melatonin and erythrocyte PMRS activity

The PMRS activity in rat erythrocyte increases (105.92 %) significantly ($p < 0.05$) in the CLE group corresponding to the control. Non-significant reduction has been found in both CDE Group (28.93%) and Group (II) melatonin treated rats (31.81%) as compared to control group. While significant ($p < 0.05$) reduction (41.16%) in the PMRS activity has been observed to the melatonin treated Group (IV) as compared to the CLE rats as shown in the Fig. 2(C).

Table 1
Effect of daily administration of melatonin on body weight, total cholesterol (TC), high density lipoprotein (HDL) and advanced glycation end products (AGEs).

	Control (C)	Control + Melatonin (C + MLT)	Continuous Light Exposure (CLE)	Continuous Light Exposure + Melatonin (CLE + MLT)	Continuous Dark Exposure (CDE)
Body weight (g)	150 ± 20	155 ± 15	218 ± 25.23*	143.33 ± 20.20$^{\#}$	160 ± 15$^{\#}$
Total cholesterol (mg/dL)	16.81 ± 3.52	15.21 ± 3.56	22.16 ± 2.92	18.08 ± 4.57	21.99 ± 3.42
HDL (mg/dL)	16.30 ± 1.10	15.34 ± 0.89	11.51 ± 1.10*	14.15 ± 1.02$^{\#}$	14.4 ± 0.79$^{\#}$
AGEs (AU/g protein)	75.66 ± 2.82	65.00 ± 3.10	90.16 ± 4.94*	70.50 ± 2.12$^{\#}$	62.16 ± 3.53*

Note: Final body weight was measured after 10 days of treatment. All values are expressed as mean ± SD of 4 animals.

[*] Significant ($p < 0.05$) when compared with the control group.

[#] Significant ($p < 0.05$) when compared to the CLE group.

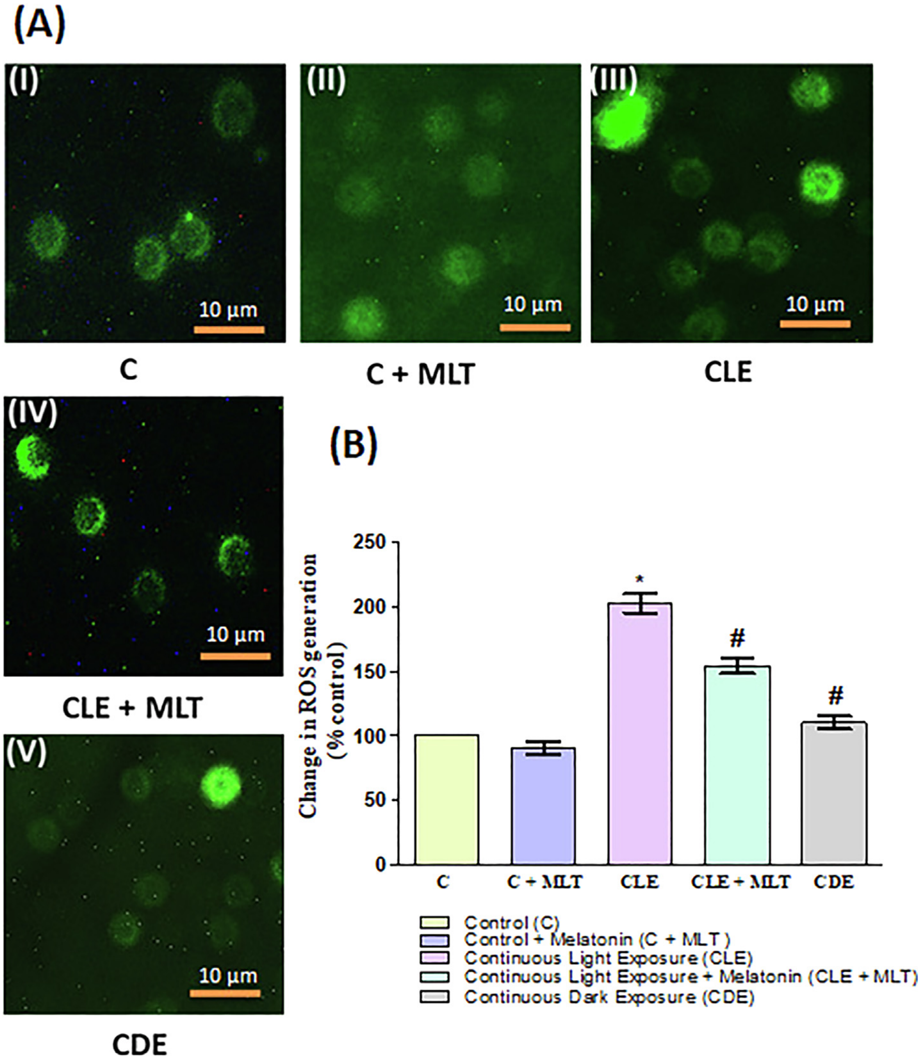

Fig. 1. Melatonin prevents changes in ROS production in continuous light exposed (CLE) and continuous dark exposed (CDE) rats. (A) Fluorescence microscopic photographs showing intracellular ROS in erythrocytes. (B) Spectrofluorimetric analysis of intracellular ROS in rat erythrocytes. Values are expressed as mean ± SD (n = 4). $^{*}p < 0.05$ when compared to Control, $^{\#}p < 0.05$ when compared to CLE rats.

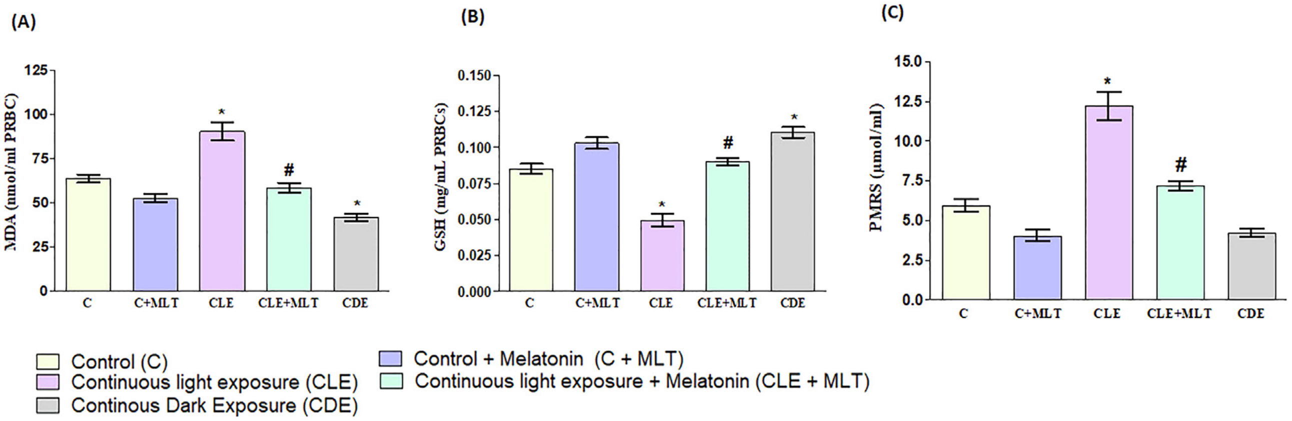

Fig. 2. Melatonin modulates biomarkers of oxidative stress in erythrocytes of continuous light exposed (CLE) and continuous dark exposed (CDE) rats. (A) Lipid peroxidation in terms of MDA (nmol/ml PRBCs), and (B) Reduced GSH (mg/ml PRBCs) content as measure of antioxidant level, and (C) PMRS (µmol ferrocyanide/ml PRBC/30 min). Values are expressed as mean ± SD (n = 4). $^{*}p < 0.05$ when compared to control, $^{\#}p < 0.05$ when compared to light exposed rats.

3.6. Melatonin supplementation and plasma antioxidant status

Anti-oxidative capacity of plasma measured in terms of FRAP, as depicted in Fig. 3(A), was reported to fall (15.89%) significantly ($p < 0.05$) in CLE rats whereas increased (10.95%) significantly ($p < 0.05$) in CDE group of rats as compared to the control. There is a non-significant increase (9.01%) in FRAP level in melatonin treated Group (II) with respect to control. While FRAP activity has been found

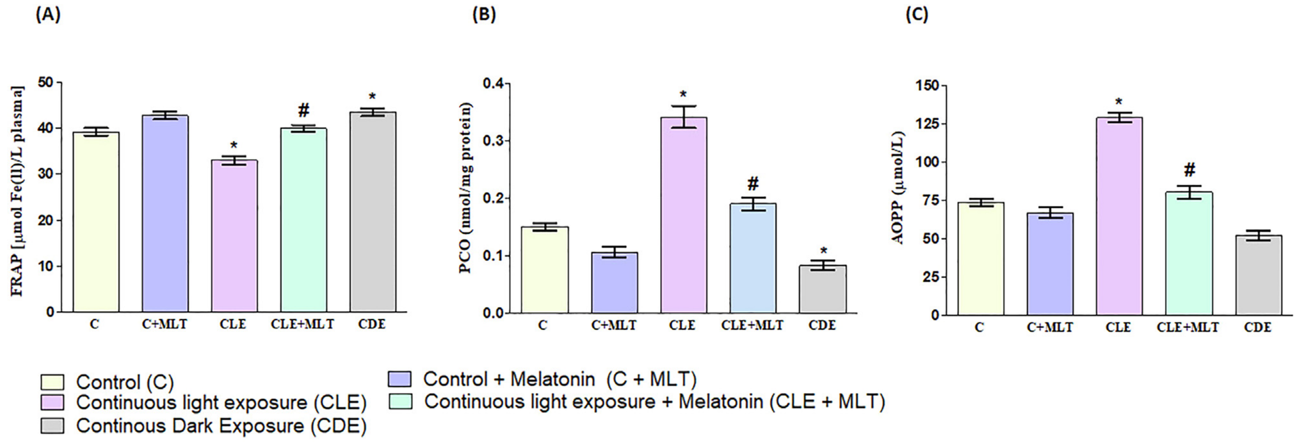

Fig. 3. Melatonin attenuates biomarkers of oxidative stress in blood plasma of continuous light exposed (CLE) and continuous dark exposed (CDE) rats. (A) FRAP (μmol Fe (II)/l plasma) measured as total antioxidant potential of plasma, (B) Protein oxidation marker measured as protein carbonyl (PC) level (nmol/mg protein), and (C) AOPP (μmol/L). Values are expressed as mean ± SD (n = 4). $^{*}p < 0.05$ when compared to control, $^{\#}p < 0.05$ when compared to light exposed rats.

significantly ($p < 0.05$) increased (21.11%) in melatonin supplemented Group (IV) as compared to CLE Group.

3.7. Melatonin and plasma protein oxidation levels

The results of protein oxidation, measured in terms of protein carbonyl (PCO) and advanced oxidation protein products (AOPP) in the plasma of rats is shown in Fig. 3(B) and (C) respectively. A significant ($p < 0.05$) increase in PCO (127.33%) and AOPP (75.07%) content was found in the rats exposed to continuous light in comparison to the control group. Melatonin supplemented Group (II) was found to be decrease (29.33% and 9.17%) non-significantly in the oxidation level of both PCO and AOPP respectively. PCO level in the CDE Group of rats was found to be decreased (44.66%) significantly ($p < 0.05$) while AOPP level found to be decrease (29.34%) non-significantly in the CDE group as compared to the control. Both PCO and AOPP were found to be reduced (44.28% and 37.78%) significantly ($p < 0.05$) in melatonin treated Group (IV) correspond to the CLE group.

4. Discussion

Artificial light pollution and use of electronic devices at night is an important contributing factor in circadian rhythm disruption (Vinogradova and Anisimov, 2013) which makes a barrier to the adjustment of internal rhythms with environmental light-dark cycle (Ikeno and Yan, 2016). Under prolonged photoperiods of artificial light and dark and the resultant loss of the circadian profile of the melatonin hormone (Dauchy et al., 2014), the function of the cell is severely compromised by excessive generation of the ROS/RNS species (Kleszczyński et al., 2016; Reiter et al., 2002). Circadian disrupted model of rat associated with modified redox state could be due to both rapid enhancement in the ROS generation and insufficiency of antioxidant defense. These processes govern the deleterious effects on proper functioning of the biological clock. Exogenous supplementation of melatonin has a restoring effect on the redox balance through suppression of ROS. Similarly increased melatonin synthesis in continuous dark exposed rat (CDE) also shows rescue from altered redox status.

The gain in the body weight under prolonged condition of artificial light and the disturbed sleep-wake cycle is frequently explained as a result of chronically altered feeding rhythm in both nocturnal and diurnal organisms. Significantly supplementation of melatonin results in normalization of the extra body weight gain without affecting food intake (Hussein et al., 2007; Nduhirabandi et al., 2010). The effect of melatonin promoting weight loss may be attributable to an increase in energy expenditure, especially by brown adipose tissue (BAT) (Tan et al., 2011). Although melatonin may also promote physical activity or cause an increase in the BMR, however, we did not observe any changes in physical activity during melatonin treatment.

Our study also demonstrated that the weight gain promoting effect of continuous light concomitantly enhances circulating plasma cholesterol level, a reduction in the circulating cholesterol level was observed in melatonin supplemented rats. Previous studies have also demonstrated the hypolipidemic effects of melatonin with reduction in the elevated levels of cholesterol, low density lipoproteins (LDL) and triglycerides (TG) in plasma (Maldonado et al., 2012). Supplementation of melatonin for several weeks is reported to suppress high levels of circulating cholesterol in adult rats and hamsters by decreasing cholesterol synthesis and augmenting endogenous cholesterol clearance mechanisms (Subramanian et al., 2007).

Previous studies have demonstrated that light emitted from LED bulb is directly responsible for massive enhancement in ROS generation (Nakashima et al., 2017). Both supplemented and endogenously synthesized melatonin act towards the removal of free radicals from the system by undergoing molecular rearrangement (Chahbouni et al., 2017). The effectiveness of melatonin to neutralize excess ROS is attributed to the generation of cascade of metabolites of melatonin which itself acts as potent antioxidant. This leads to a broad spectrum antioxidant function of melatonin, significantly a single molecule of melatonin has the capacity to neutralize up to 10 radical species before the last metabolite is excluded from the body (Johns and Platts, 2014).

Blood plasma consists of several enzymatic and nonenzymatic antioxidants which may be quantified by measuring ferric reducing ability of plasma (FRAP), widely used as an index of plasma antioxidant reserve. Previous reports show that FRAP value decrease with alteration in the redox homeostasis (Goraca, 2004). Oral administration of melatonin, at dawn, significantly enhance the capacity of plasma to reduce Fe^{+3} to Fe^{+2}. Basically melatonin binds to Fe^{+3} and transforms it to a more biologically consumable form of iron (Fe^{+2}) Our study demonstrated the reduction in the FRAP ability during continuous light. The fact that both oral supplementation and naturally synthesized melatonin has the capability to restore FRAP index correlates with the findings reported by Piechota et al. (2010).

MDA and 4-hydroxynonenal (HNE) are the major aldehydic products of the lipid peroxidation The demonstrated increment in the MDA concentration subsequent to continuous light points to an intensified lipid peroxidation taking place in a light exposed group. Published reports emphasize an increased level of MDA in the erythrocyte during photophase for all the light spectra (Shin et al., 2011). Lipid peroxidation results in decreased membrane fluidity (Spiteller, 2001), increased nonspecific permeability and inactivation of some membrane enzymes which may have stimulating effect on the process of aging and the development of advanced age diseases. Both supplemented and endogenous melatonin has been found to decrease MDA level corroborating our results (Piechota et al., 2010). In vitro studies have also confirmed an inhibitory effect of melatonin on lipid peroxidation (Sewerynek et al., 1995). The inhibitory effect of the hormone on lipid peroxidation is supposed to result from the ability of melatonin to extinguish free radicals initiating lipid peroxidation or, possibly, due to factors not yet recognized (Reiter et al., 2003).

Reduced glutathione (GSH) is the most influential cellular antioxidant molecule which protects various proteins in the erythrocytes such as spectrin. There is an active participation of GSH to confer cellular defense against reactive oxygen and nitrogen species (RONS). The capability of melatonin to upregulate antioxidative activity by stimulating antioxidative system indirectly is of great importance (Tomás-Zapico and Coto-Montes, 2005). Our observation of an increase in oxidative stress in erythrocytes during chronic light condition is further substantiated with reduction in the GSH content. Melatonin may affect the GSH level by inducing expression of Ỵ-glutamylcysteine synthetase, an enzyme limiting tripeptide synthesis (Urata et al., 1999).

PMRS is known to play vital role in the maintenance of cellular redox homeostasis (de Grey, 2005). Erythrocyte PMRS activity gets elevated to provide a compensatory mechanism for neutralization of higher ROS production and resultant oxidative stress during aging (Kim et al., 2016; Rizvi et al., 2006). In particular PMRS operates through the maintenance of NAD $(P)^+$/NAD (P) H ratio and is active in cell under anaerobic condition (Merker et al., 2002). In the given study, PMRS level increased dramatically in light stressed condition which has been compensated in the presence of melatonin highlighting its tremendous antioxidant potential. To the best of our knowledge this is the first report of an effect of in vivo effect of melatonin on the PMRS activity. In vitro study of diurnal pattern of melatonin is reported to cause an elevated level of PMRS in the dark condition (Chakravarty and Rizvi, 2012).

PCO is one of the prominent markers of oxidative stress. Previous findings have established an increase in plasma protein carbonyl during oxidative stress in humans and rats (Pandey and Rizvi, 2010). Several mechanisms such as peptide backbone fragmentation, extraction of hydrogen from alpha carbon atom or an oxidative attack on several amino acid side chains, and adduct formation between some amino acids and MDA play a role in the formation of PCO. It is also generated by glycation or glycoxidation of lysine amino groups (Dalle-Donne et al., 2006). Our observation of a decrease in rat plasma PCO content subsequent to melatonin treatment is corroborated by a study of normalization of PCO content induced by oxaliplatin compensated with melatonin (Chaudhary and Parvez, 2017; Waseem et al., 2016). Advanced oxidation protein products (AOPP) are generated as a consequence of free radical attack on proteins and is frequently used as a biomarker of the oxidative stress (Selmeci et al., 2005). AOPP might be formed due to reaction of chlorinated oxidants with plasma proteins under stressful condition. Gradual increase in AOPP level has already been reported previously from our laboratory during aging in humans (Pandey and Rizvi, 2010) and in rats (Chaudhary et al., 2017; Garg et al., 2017).

Serum advanced glycation end products (AGEs) linked fluorescence which is directly correlated with hyperglycemic and carbonyl stress has the potential to be developed as a surrogate biomarker for oxidative stress (Gopal and Indira, 2010). Both supplemented and naturally synthesized melatonin attenuates the rise in AGEs in continuous light exposed rats and improves antioxidant status through trapping of interaction with dicarbonyl compounds generated through glycation process.

5. Conclusion

Although artificial light can be used to increase human productivity, its ill effects on physiology can be extremely stressful. The modification of circadian clock may lead to severe alteration in redox balance. Our results provide enough evidence explaining that a deficit of the pineal hormone affects antioxidative homeostasis in rats which leads to the alteration of antioxidative defense and development of prooxidative processes. The efficacy of melatonin to restore altered redox mechanisms creates the possibility of melatonin to be used in conditions involving circadian disruption and several neurological and physiological situations which involve redox imbalance.

6. Disclosure of interests

The authors declare that there are no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.04.016.

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