Pharmacological Intervention in dim light induced changes in circadian rhythm

Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

By

Prabha Rajput

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI– 221005
INDIA

Roll No. 17161007

2023

Chapter 6

Summary and Conclusion

The key findings of our thesis are that chronic exposure to dim artificial light disrupt both the central (SCN) and the peripheral (liver) clocks. These disrupted circadian clocks lead to dysregulated mitochondrial functions (like RCR, ATP synthesis, ratio of mtDNA/DNA) which shows robust circadian rhythmicity under normal light-dark cycle (LD12:12) over 24h cycle. Chronic dim LL acts as stress to animals and altered several key circadian parameters like amplitude, mesor and acrophase (time at which maximum level) of the estimated parameters like RCR, ATP ratio, ratio of mtDNA/DNA, CORT etc., causing arrhythmicity. Such arrhythmicity dysregulated the core clock genes and key mitochondrial enzyme such as GSH. This altered the mitochondrial bioenergetics that may lead to several metabolic disorders like obesity, diabetes etc. Some of the key mitochondrial functions and CORT levels were quantified of a 24-h cycle, and other key parameters like melatonin, leptin, ghrelin, glutathione (GSH) were evaluated at the maximum responsive zone over a 24-h period, specifically at ZT/CT15 under LD and chronic dim LL conditions.

Chronic dim light act as a stressor by increasing the CORT and decreasing the melatonin level, thus increasing the free radical load in the brain leading to oxidative stress. During chronic dim LL, at maximum responsive zone of a 24-h cycle under LD and LL (ZT15/CT15), NAC reduced the elevated CORT level, and stabilized the endogenous melatonin level. Further, it also alleviated mitochondrial oxidative stress by replenishing the GSH levels. NAC improved dim light induced changes in mitochondrial bioenergetics and DNA copy number. Such improvement in

mitochondrial function caused re-synchronization of the circadian rhythms of melatonin, CORT, RCR and mitochondrial number as estimated at ZT/CT 15 under dim light exposure. Insufficiency of GSH caused glutamate-induced excitotoxicity which was found to be alleviated by NAC.

Liver, which is a key organ involved in maintaining glucose homeostasis, has its own clocks (peripheral clocks). It is rich in mitochondria and is involved in glucogenesis and glycogenolysis, depending upon the energy balance of the cell. Untimed fasting and feeding cycle disturbs the glucose homeostasis of the cell. Chronic dim light leads to altered feeding-fasting rhythm and disturbs the liver clocks causing obesity and several metabolic changes in mitochondria. This resulted in reduced GSH and mitochondrial enzyme i.e., succinate dehydrogenase (SDH) and caused disruptions in the levels of leptin and ghrelin, which regulates the intake of food. Such alteration in the above hormones can disrupt the peripheral clocks and can alter the eating behavior. NAC at various doses improved the GSH and SDH in the liver as well as leptin and ghrelin. Hence, NAC can be used as a potential chrono-biotic molecule for treating disrupted circadian disorders.

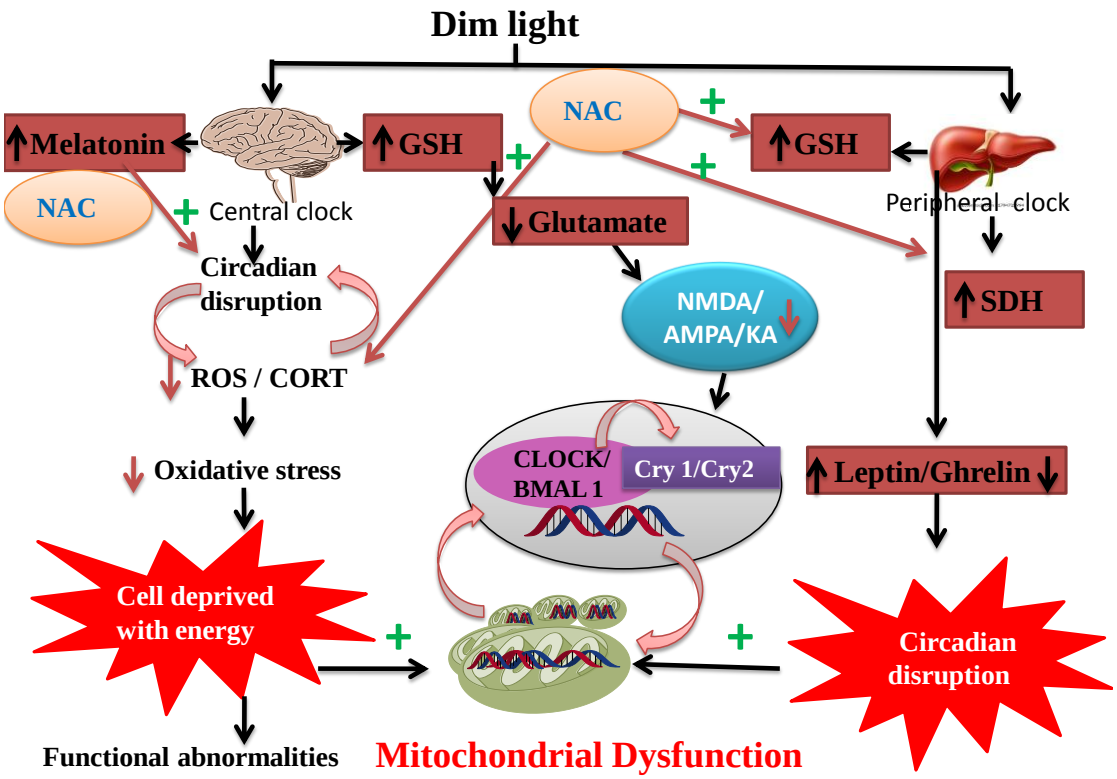

Figure 6.1 *Summary and conclusion of the study: arrow downwards represents decrease; arrow upwards represents increase;* ✚ *symbol indicates improve*ment

6.1 Important outcomes

- Mitochondrial respiration and mitochondrial DNA copy number follows the rhythmic phenomena in normal light dark condition and becomes arrhythmic in dim light condition evaluated at eight time points within 24 h.

- The maximum activity of mitochondrial respiration, and mitochondrial DNA copy number was found during night at 9 pm (ZT 15), 3 h after switching off the light.

- N-acetylcystiene protects dim light-induced mitochondrial dysfunction at various doses in SCN and liver.

- N-acetylcystiene improved mitochondrial function as well as circadian rhythm disruption at various doses.

6.2 Limitation of the study

- The clock gene measured at maximum activity time point i.e. ZT 15 only.
- Continuous dim LL condition has been studied; other light condition could be considered.
- White LED light has been used to this study; blue light could be considered for further study.
- This study does not measure phase delayed and phase advanced condition.

6.3 Future studies

- In the present study, clock genes expression has been evaluated at one time point (ZT/CT 15) where mitochondrial respiration, ratio of mtDNA/nDNA and corticosterone level was found to be the highest. Therefore, a future study evaluating the clock genes expression over the entire 24 h cycle can be performed to know their day-night expression pattern.
- In the present study we evaluated the effect of continuous dim light (24:00 h) on mitochondrial respiration and function in the central (SCN) and peripheral (Liver) clock. Further studies can be performed which will evaluate the effect of continuous dim light exposure on other peripheral clocks of heart, lungs, and kidney.
- In the current study, we found that NAC up-regulated the clock genes expression in the central clock (SCN). Therefore, future studies measuring the clock genes in the peripheral clocks can be performed.