# Pharmacological Intervention in dim light induced changes in circadian rhythm


**Thesis submitted in partial fulfillment for the Award of Degree**

## Doctor of Philosophy

**By**

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It is certified that the work contained in the thesis titled "**Pharmacological intervention in dim light induced changes in circadian rhythm.**" has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

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## DECLARATION BY THE CANDIDATE

I, **Ms. Prabha Rajput**, certify that the work embodied in this Ph.D. thesis is my own bonafide work and carried out by me under the supervision of **Prof. Sairam Krishnamurthy** from **July, 2017 to July, 2023** at the **Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi.** The matter embodied in this Ph.D. thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged and given credit to the research workers wherever their works have been cited in my work in this thesis. I further declare that, I have not willfully copied any other's work, paragraphs, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, theses, etc., or available at websites and have not included them in this Ph.D. thesis and have not cited as my own work.

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**Signature of the Student**

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It is certified that the above statement made by the student is correct to the best of our knowledge.

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## ACKNOWLEDGEMENT

Joys on successful completion are always cherishing and everlasting. It gives the feeling of completeness on looking back over the journey and remembering all those friends and family who have helped and supported me along this long but fulfilling path. I owe my gratitude to the almighty **'*Lord Shiva'*** for blessings and making things all right in place. ***Bharat Ratna Mahamana Pandit Madan Mohan Malaviya Ji,*** Founder of BHU, for providing me such a divine platform.

Words cannot express my gratitude for my family: My mother, ***Mrs. Minti Rajput,*** and my Father, ***Mr. Jujhar Singh Rajput,*** for supporting me spiritually throughout writing this thesis and my life in general.

At this moment of accomplishment, firstly of all, I pay homage to my Ph.D. supervisor ***Prof. Sairam Krishnamurthy*** (Professor of Pharmacology), for giving me an opportunity to work in the field of neuropharmacology, the research area I love to work with; I am also grateful to him for grooming me not only to conduct independent research but also for acquainting me with other areas affiliated with scientific pursuits. His wide knowledge and logical way of thinking have been a great value to me. This work would not have been possible without his guidance, support and encouragement. Under his guidance, I successfully overcame many difficulties and learned a lot. His unflinching courage and conviction will always inspire me, and I hope to continue to work with his noble thoughts.

I thank my research progress evaluation committee members, ***Dr. P.K. Nayak***, Department of Pharmaceutical Engineering and Technology, and ***Dr. Abha Mishra,*** Department of Biochemical engineering, for their valuable suggestions and comments during my Ph.D. tenure.

I thank Head of the Department (HOD) **Prof. Siva Hemalatha** and former HOD**, Prof. B. Mishra, Prof. S.K. Singh, Prof. Sanjay Singh and Prof. S.K. Shrivastava** for their support.

It is my extreme privilege to gratuitously convey my special thanks to all the faculty members of the Department, ***Prof. S. Hemlatha, Dr. A.K. Srivastava, Dr. Senthil Raja, Dr. A.N. Sahu, Dr. S.K. Mishra, Dr. Ruchi Chawla, Dr. Ashok Kumar Maurya, Dr. M.S. Muthu, Dr. G.P. Modi, Dr. P.K. Nayak, Dr. A.K. Agrawal, Dr. S.K. Jain and Dr. Vinod Tiwari, Dr. Rajnish, Dr. Deepak Kumar, Dr. Dinesh Kumar, Dr. Jairam Meena, and Dr.A.Khatri*** for their kind cooperation and valuable suggestions throughout the research work.

My sincere thanks to ***Mr. Ram Jiyawan, Mr. Yashwant Singh, Mr. Atul Kumar, Mr. Anand, Mr Chotelal, Mr. Virendra Kumar, Mr. Nandlal, Mr. Madanlal, Mr. Ram Hriday Pathak, Mr. Akhila Nand Upadhyay, Mr. Arun Kumar, Mr. Md. Jameel, Mr. Sunil Kumar Singh, ,*** and all other non-teaching staff of the department who had provided me all the necessary support while needed.

I am thankful to my Seniors/labmates/juniors, ***Dr. Sukesh, Dr. Pankaj, Dr. Akanksha, Mr.Santosh Mr. Ramakrishna, Mr. Qadir, Ms. Pratigya, Ms. Shreyasi, Ms. Neha, Ms. Asha, Mr. Gajendra, Mr. Aquib, and Mr. Neeraj,*** for stimulating discussions, for the sleepless nights we were working together before deadlines, and all the fun we have had in the last Six years. I would also like to express my gratitude to all those who supported me directly or indirectly for the study.

I would also like to thank ***Dr. Dhananjay Kumar*** for his help during my Ph.D. period. A special thanks ***to Madhusudan and Tavarez*** for their assistance during my experimental work.

Finally, I am highly indebted to the almighty for showering immense blessing in gifting me my beloved parents, family, and friends.

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ADP | - | Adenosine Di-Phosphate |
| ATP | - | Adenosine Triphosphate |
| BSA | - | Bovine Serum Albumin |
| CORT | - | Corticosterone |
| CT | - | Circadian Time |
| DMSO | - | Dimethyl sulfoxide |
| EGTA | - | Ethylene Glycol-bis (β-aminoethyl ether)-N, N, N', N'-Tetra acetic Acid |
| ETC | - | Electron transport chain |
| FCCP | - | Carbonyl Cyanide 4-(trifluoromethoxy) Phenylhydrazone |
| HEPES- 4 | - | (2-HydroxyEthyl)-1-Piperazine Ethane Sulfonic acid |
| HPLC | - | High-performance liquid chromatography |
| $KH_2PO4$ | - | Potassium Phosphate Monobasic Anhydrous |
| LD | - | Light Dark (12:12 h) |
| LL | - | Continuous dim light (24:00 h) |
| MET | - | Metformin |
| $MgCl_2$ | - | Magnesium Chloride |
| MPTP | - | 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine |
| NAD | - | Nicotinamide adenine dinucleotide |
| NADH | - | Nicotinamide adenine dehydrogenase |
| OXPHOS | - | Oxidative phosphorylation |
| Q10 | - | Coenzyme Q10 |

| | | |
|---|---|---|
| RCR | - | Respiratory Control Rate |
| RSV | - | Resveratrol |
| RT | - | qPCR- Real-Time Polymerase Chain Reaction |
| SCN | - | Supra-Chiasmatic Nucleus |
| ZT | - | Zeitgeber Time |
| CPCSEA | - | Committee for the Purpose of Control and Supervision of Experiments on Animals |
| IAEC | - | Institutional Animal Ethical Committee |
| TMRM | - | Tetra-methyl rhodamine methyl ester |
| NAC- N | - | acetylcysteine |
| Mel | - | Melatonin |
| BMAL1 | - | Brain and muscle ARNT-Like 1 |
| HPLC | - | High-performance liquid chromatography |
| ANOVA | - | One-way analysis of variance |
| SDH | - | Succinate dehydrogenase |
| CLOCK | - | Circadian locomotor output cycles kaput |

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# PREFACE

The thesis research work entitled “Pharmacological intervention in dim light induced changes in circadian rhythm." assessed the effect of continuous dim light on the rhythmicity of mitochondrial function in the superchiasmatic nuclei (SCN). Further, we evaluated the role of mitochondria in normal circadian rhythm (light dark; LD 12:12 h) and disrupted circadian rhythm (continuous dim light; LL 24:00 h). Further, first time we are showing there is a rhythmic pattern in mitochondrial bioenergetics and mitochondrial DNA in LD condition over the 24-h in along with corticosterone and the rhythmicity was lost in the LL condition. Therefore, we sought to the effect of mitochondrial modulator N-acetylcysteine (NAC) on dim light induced mitochondrial changes in central and peripheral tissue clock. Moreover, we evaluated the NAC effect on different circadian clock genes, endogenous melatonin and hunger hormone leptin and ghrelin which was attenuated at different doses with NAC in mice exposed to dim light. Furthermore, there are several pharmacological agents like metformin that are promising to have a mitochondrial potential or inhibitory effect there is no screening method to test their activity on mitochondria. Hence, we have developed a robust economic *ex-vivo* method for mitochondrial bioenergetics using mitochondrial modulators.

The whole work has been compiled into six chapters: **Chapter 1** introduces the topic and its importance. **Chapter 2** Effect of normal and disrupted circadian rhythm on the mitochondria. **Chapter 3** Effect of N-acetycysteine on mitochondria dysfunction induced by disrupted circadian rhythm in central clock. **Chapter 4** documents the effect of N-acetycysteine on mitochondria dysfunction induced by disrupted circadian rhythm in peripheral clock. **Chapter 5** Novel *Ex-vivo* method development for the evaluation of mitochondrial modulators and **Chapter 6** summarizes the entire study with the conclusion and important outcomes.