CERTIFICATE

It is certified that the work contained in the thesis titled "**Pharmacological Evaluation of Orexin Antagonist in the Experimental Model of Post-Traumatic Stress Disorder**" by **Mr. Santosh Kumar Prajapati** 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.

Date:

Place: IIT (BHU), Varanasi

Prof. Sairam Krishnamurthy
(Supervisor)

DECLARATION BY THE CANDIDATE

I, **Santosh Kumar Prajapati**, 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, 2015 to January, 2021** 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.

Date:

Place: IIT (BHU), Varanasi **Santosh Kumar Prajapati**

CERTIFICATE BY THE SUPERVISOR AND HEAD OF THE DEPARTMENT

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

Prof. Sairam Krishnamurthy
(Supervisor)

Prof. Sushant Kumar Shrivastava
(Head of the Department)

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis : Pharmacological Evaluation of Orexin Antagonist in the Experimental Model of Post-Traumatic Stress Disorder

Candidate's Name : Mr. Santosh Kumar Prajapati

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (Banaras Hindu University), Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the **"*Doctor of Philosophy*".**

Date:

Place: IIT (BHU), Varanasi **Santosh Kumar Prajapati**

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. Lahasiya Devi,*** and my Father, ***Mr. Munnilal Prajapati,*** 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. Jeyakumar Kandasamy,*** Department of Chemistry, for their valuable suggestions and comments during my Ph.D. tenure.

I warmly thank ***Dr. Subash C. Gupta*** and ***Mr. Vipin rai*** Department of Biochemistry, Institute of Science, Banaras Hindu University, for providing IHC and Western blot facility. I would also like to express my thanks to BHU's Interdisciplinary School of Life Sciences for providing microtome for tissue sectioning.

I thank Head of the Department (HOD) ***Prof. Sushant Kumar Shrivastava*** and former HOD**, Prof. B. Mishra, Prof. S.K. Singh** and **Prof. Sanjay Singh** 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*** for their kind cooperation and valuable suggestions throughout the research work.

My sincere thanks to ***Mr. Ram Jiyawan, Mr. Yashwant Singh, 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, Mr. Anand,*** and all other non-teaching staff of the department who had provided me all the necessary support while needed.

I am thankful to my labmates, ***Dr. Dhananjay, Sukesh, Pankaj, Akanksha, Ramakrishna, Qadir, Prabha, Pratigya, Shreyasi, Smriti, Neha, Gajendra, Aquib, Vishal, and Asha,*** for stimulating discussions, for the sleepless nights we were working together before deadlines, and all the fun we have had in the last five 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. Puneet*** for his cooperation 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.

Date:

Place: IIT(BHU), Varanasi **Santosh Kumar Prajapati**

TABLE OF CONTENTS

LIST OF FIGURES & TABLES

LIST OF ABBREVIATIONS & SYMBOLS

5-HIAA: 5-Hydroxyindoleacetic Acid

5-HT: Serotonin

ACh: Acetylcholine

AChE: Acetylcholinesterase

ACTH: Adreno Corticotrophin Hormone

AMY: Amygdala

ANOVA: Analysis of Variance

BNST: Bed Nucleus of the Stria Terminalis

CMC: Carboxymethyl Cellulose

CORT: Corticosterone

CRF: Corticotropin-Releasing Factor

CRF-R1: Corticotropin-Releasing Factor-Receptor1

DA: Dopamine

DRN: Dorsal Raphe Nucleus

Drp-1: Dynamin-Related Protein

DSM-V: Diagnostic and Statistical Manual of Mental Disorders

ECD: Electrochemical Detector

EDS: Extra-Dimensional Shift

EPM: Elevated Plus Maze

FDA: Food and Drug Administration

FS: Foot Shock

FST: Forced Swim Test

GC: Glucocorticoid

GR: Glucocorticoid Receptor

HIP: Hippocampus

HPA-Axis: Hypothalamic–Pituitary–Adrenal-Axis

HPLC: High Performance Liquid Chromatography

ICV: Intracerebroventricular

IDS: Intra-Dimensional Shift

LC: Locus Coeruleus

MANOVA: Multivariate Analysis of Variance

MAO: Monoamine Oxidase

Mfn-1,2: Mitofusin-1,2

MMP: Mitochondrial Membrane Potential

MR: Mineralocorticoid Receptor

MTFP-1: Mitochondrial Fission Process

m-TOR: Mammalian Target of Rapamycin

mTORC-1: Mammalian Target of Rapamycin Complex-1

NE: Norepinephrine

Opa-1: Optic Atrophy-1

OX1-R: Orexin Receptor-1

OX2-R: Orexin Receptor-2

PAX: Paroxetine

PFC: Prefrontal Cortex

PTSD: Post-traumatic Stress Disorder

PVN: Paraventricular Nucleus

RAPA: Rapamycin

RCR: Respiratory Control Ratio

ROS: Reactive Oxygen Species

SERT: Sertraline

SSRIs: Selective Serotonin Reuptake Inhibitors

SUVO: Suvorexant

UV: Ultraviolet

VTA: Ventral Tegmental Area

α-7nAChR: Alpha-7nicotinic Acetylcholine Receptor

%: Percent

cm: Centimeter

D: Day

g: Gram

h: Hour

kg: Kilogram

mA: Milliampere

mg: Milligram

min: Minute

mM: millimole

nm: Nanometer

pg: Picogram

PO: Per Oral

sec: Second

μL: Microlitre

PREFACE

The thesis research work entitled "Pharmacological Evaluation of Orexin Antagonist in the Experimental Model of Post-Traumatic Stress Disorder" assessed the involvement of the orexinergic system in the stress re-stress (SRS) model of PTSD and its pharmacological effects on PTSD-like symptoms. Further, during PTSD, there is a negative alteration in cognitive function due to the fear response of traumatic events, which is interpreted as cognitive inflexibility. Cognitive inflexibility is one of the clinically observed major symptoms of PTSD, together with fear and anxiety. Therefore, we evaluated cognitive inflexibility for the first time in the SRS paradigm. Moreover, we have evaluated the alteration of mitochondrial dynamics in the SRS model as an alternative pathology of PTSD and the role of orexinergic antagonism on mitochondrial dynamics in rats exhibiting PTSD-like symptoms.

The whole work has been compiled into **five chapters**: **Chapter 1** introduces the topic and its importance. **Chapter 2** reports the anti-PTSD potential of orexin antagonist in SRS exposed rats. **Chapter 3** reports the validation of cognitive inflexibility in the SRS model of PTSD. **Chapter 4** documents the effect of orexin antagonist on mitochondrial dynamics in rats exhibiting PTSD-like symptoms. **Chapter 5** summarizes the entire study with the conclusion and important outcomes.