Pharmacological evaluation of selected atypical antipsychotics in an experimental model of Post-Traumatic Stress Disorder (PTSD)

THESIS SUBMITTED FOR THE AWARD OF THE DEGREE
OF
Doctor of Philosophy

Submitted By

N. Ranga Reddy

M. Pharm

Supervisor

Prof. Sairam Krishnamurthy

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)

Enrolment No. 295574 **Roll No. 10621EN004**

Dedicated to

my PARENTS

भारतीय प्रौद्योगिकी संस्थान
काशी हिन्दू विश्वविद्यालय
IIT INDIAN INSTITUTE OF TECHNOLOGY
BANARAS HINDU UNIVERSITY

Department of Pharmaceutical Engineering & Technology

Undertaking from the Candidate

I, **N. Ranga Reddy**, declare that the work embodied in this Ph.D. thesis is my own bonafide work carried out by me under the supervision of **Prof. Sairam Krishnamurthy** for a period of about 07 years 10 months from August, 2010 to June, 2018 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.

Date:

Place: Varanasi **(N. Ranga Reddy)**

ANNEXURE- E

CANDIDATE'S DECLARATION

I, **N. Ranga Reddy**, certify that the work embodied in this PhD thesis is my own bonafide work carried out by me under the supervision of **Prof. Sairam Krishnamurthy** for a period of about 07 years 10 months from August 2010 to June 2018 at the Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this PhD thesis has not been submitted for the award of any other degree/diploma.

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

Date:
Place: Varanasi **(N. Ranga Reddy)**

Certificate from the Supervisor/Co-Supervisor/Head of the Department

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

(Prof. Sairam.K)
Supervisor

Head of the Department

ANNEXURE- F

COURSE/COMPREHENSIVE EXAMINATION COMPLETION CERTIFICATE

This is to certify that **Mr. N. Ranga Reddy,** a bonafide research scholar of this Department, has successfully completed the course work/comprehensive examination requirement, which is a part of his Ph.D. programme.

Date:

Place: Varanasi **(Head of the Department)**

भारतीय प्रौद्योगिकी संस्थान
काशी हिन्दू विश्वविद्यालय
IIT INDIAN INSTITUTE OF TECHNOLOGY
BANARAS HINDU UNIVERSITY
Department of Pharmaceutical Engineering & Technology

ANNEXURE- F

PRE-SUBMISSION SEMINAR COMPLETION CERTIFICATE

This is to certify that **Mr. N. Ranga Reddy,** a bonafide research scholar of this Department, has successfully completed the Pre-submission seminar requirement on the topic***"Pharmacological evaluation of selected atypical antipsychotics in an experimental model of Post-Traumatic Stress Disorder (PTSD)"***on **28.06.2018**, which is a part of his Ph.D. programme.

Date:

Place: Varanasi **(Head of the Department)**

ANNEXURE- G

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: "Pharmacological evaluation of selected atypical antipsychotics in an experimental model of Posttraumatic Stress Disorder (PTSD)"

Candidate's Name: N. Ranga Reddy

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 Ph.D. degree.

Date: **(N. Ranga Reddy)**

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for the author's personal use provided that the source and the Institute's/University's copyright notice are indicated.

Acknowledgement

The overwhelming joy of the successful completion is always cherishing and everlasting. It gives me 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 for the love and support of my father, **Shri. N. Jagannatha Reddy** and mother, **Smt. N. Hanumanthamma,** in taking up the path of education. Besides, I also cherish the love and support of my family, who stood with me throughout the phase of completion.

I bow down at feet of **Lord Kashi Viswanath** for providing the strength in pursuing the education at Varanasi.

At this moment of accomplishment, firstly of all, I pay homage to my Ph.D. supervisor **Dr Sairam Krishnamurthy,** Professor of Pharmacology, for giving an opportunity to work in the field of neuropharmacology. I am also grateful to him for his constant support and guidance in the completion of my research and other scientific pursuits. This work would not have been possible without his extensive knowledge, experience 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 also take this opportunity to sincerely acknowledge the **Council of scientific and industrial research, New Delhi and Indian Institute of Technology, BHU,** for providing financial assistance in the form of a Senior Research Fellowship, which helped to perform the work comfortably. I thank **Prof. Sanjay Singh (Head of the Department), IIT (BHU), Prof. B. Mishra, Prof. S.K.Singh, Prof. S.K. Srivastava, Mr. A.K. Srivastava, Prof. (Mrs.) S.**

Hemalatha, Dr. Vikas Kumar, Abha Mishra (School of Biochemical Eng), Dr. Senthil Raja A, Dr. Alakh N. Sahu, Dr. S.K. Mishra, Dr. Ruchi Chawla, Dr. M.S. Muthu, Dr. Prasanta Kumar Nayak, Dr. GyanPrakash Modi, Dr. Shreyans Kumar Jain, Dr. Vinod Tiwari, Dr. Ashish Kumar Agrawal, Dr. Ashok Kumar for their support on various occasions during my PhD work.

I also thank my labmates Debapriya, Santosh, Sukesh, Pankaj, Akanksha, Ramakrishna, Ritu, Millind, Rajesh and Dhananjay.

I would also like to thank Satish Thokhla, Ramoji Kosuru, Ankit Seth and all my seniors and colleagues who supported me throughout the Ph.D. period.

I feel great pleasure to thank all the non-teaching staff of the department, Nandlalji, Madanji, Virendraji, S.K.Pathakji, Md. Jameelji, Rafiqueji, Mrs. Archana Singh and ShyamliGhoshal ma'am. I would also like to express my vote of thanks to all the office staff members, Upadhyayji, Sanjeevji, Sunilji, Ram jiawanji, Yashwantji, Anand Ji and Madhu Sudan.

I beg a deep level of forgiveness from the rats that were sacrificed during my experimental work.

Finally, and most importantly, I would like to thank my wife, **Smt. A Bhargavi** for her support and encouragement. Special appreciation for my sweet son **Chaitanya Reddy** and lovely daughter **Hari Nandana**.

Table of Contents

List of Figures

List of Tables

List of Abbreviations and Symbols

%	:	Percent
±	:	Plus or minus
µl	:	Microliter
µg	:	Microgram
ng	:	Nanogram
µm	:	Micrometre
pg	:	Picogram
g	:	Gram
h	:	Hour
kg	:	Kilogram
mg	:	Milligram
mM	:	Millimolar
M	:	Molar
mL	:	Millilitre
α	:	Alpha
β	:	Beta
γ	:	Gamma
κ	:	Kappa
≥	:	Greater than or equal to
=	:	Equal to
°C	:	Degree centigrade
mmol	:	Millimoles
pH	:	Potential of hydrogen
L	:	Litre
dL	:	Decilitre
>	:	Greater
≤	:	Less than or equal to
Ca^{+2}	:	Calcium
BDNF	:	Brain-derived nerve growth factor

CREB	:	Cyclic AMP responsive element binding protein
p-ERK	:	Phosphorylated extracellular regulated kinase
ERK	:	Extracellular signal-regulated kinase
APZ	:	Aripiprazole
OLZ	:	Olanzapine
RIS	:	Risperidone
EPM	:	Elevated plus-maze
Plasma Cort	:	Plasma corticosterone
NE	:	Norepinephrine
5HT	:	Serotonin
DA	:	Dopamine
5HIAA	:	5-Hydroxy Indole acetic acid
HVA	:	Homovanilic acid
WHO	:	World Health Organization
i.v.	:	Intravenous
i.p.	:	Intraperitoneal
p.o.	:	Peroral
rpm	:	Revolutions per minute
mU	:	Milliunits
U	:	Units
∞	:	infinity
V	:	Volt
≤	:	Less than or equal to
vs	:	Versus
&	:	And
mmHg	:	Millimeter of mercury
pg	:	Picogram

PREFACE

The research work of the thesis entitled "Pharmacological evaluation of selected atypical antipsychotics in an experimental model of Post-Traumatic Stress Disorder (PTSD)" is based on the evaluation of atypical antipsychotic drugs in the treatment of PTSD with regards to their modulating effects on cell survival factors, serotonin levels and plasma corticosterone. The symptoms of PTSD are thought to persist due to the failure of the neuroadaptive mechanisms required for the extinction of fear memories. The selected antipsychotics like olanzapine (OLZ), aripiprazole (APZ), and risperidone (RIS) were found to mitigate PTSD symptoms in human studies. Further, they were found to bring about neuroadaptive changes in the brain through the modulation of cell pathway factors. Hence, they were evaluated for their effects on stress and also PTSD in rats. The whole work has been compiled into six chapters: **Chapter 1** describes the anti-stress effects of olanzapine. **Chapter 2** mentions the anti-stress effects of aripiprazole. **Chapter 3** investigated the anti-stress effects of risperidone. **Chapter 4** investigated the preclinical potential of olanzapine in stress re-stress model of rats with effects on plasma corticosterone, neurotrophic factors like brain-derived nerve growth factor (BDNF) cyclic AMP-responsive element-binding protein (CREB), extracellular regulated kinase (ERK) and caspase-3 and apoptotic enzyme.

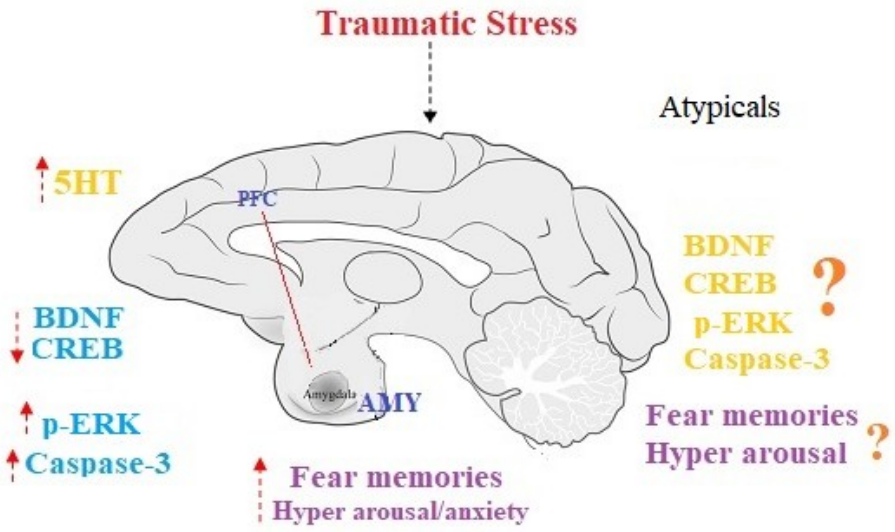
Further, the effect on serotonin was also estimated. **Chapter 5** describes the anti-PTSD potential of aripiprazole in the stress-restress model of rats with an emphasis on plasma corticosterone and neurotrophic factors. **Chapter 6** evaluated the effect of risperidone in the stress re-stress model of PTSD rats in terms of cell survival factors, plasma corticosterone and serotonin. Thus, the entire study indicates a significant therapeutic potential of selected atypical antipsychotics in the stress re-stress model of PTSD in rats.

Introduction

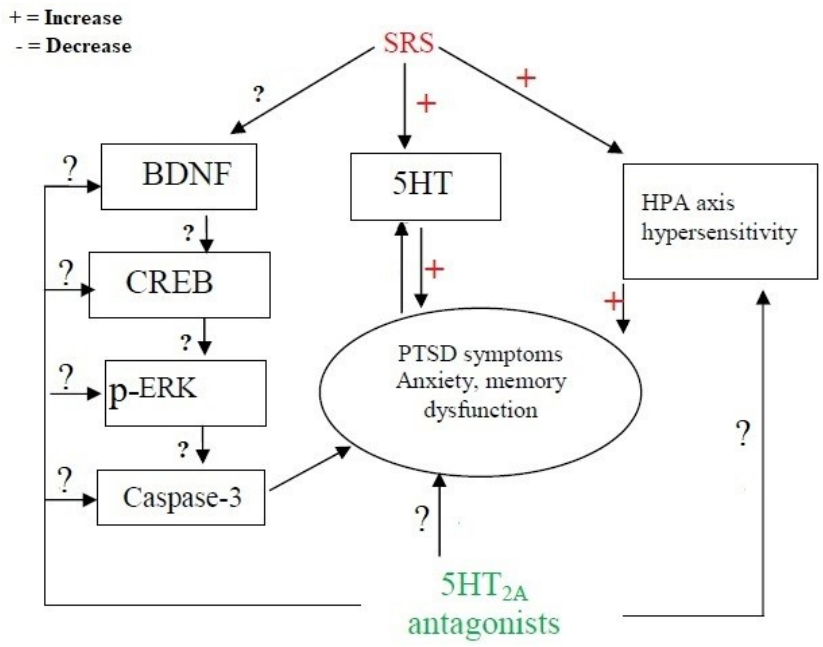
Post-traumatic stress disorder (PTSD) in one of the anxiety disorders that occurs in people who have undergone terrifying experiences. These experiences are traumatic and the symptoms can last for prolonged periods. As per NIH, PTSD occurs due to experiences like the death of loved ones, sexual assault, accidents, and natural disasters. PTSD patients show four characteristic symptoms like recurring thoughts, hyperarousal, avoidance and cognitive disturbances. In general, the symptoms of traumatic experiences last for three months in general and beyond this could be diagnosed as PTSD. However, a person with PTSD should show all of the four symptoms for at least one month. This indicates that the symptoms of PTSD persist for a long time due to a lack of adaptive mechanisms in the brain (Whitaker et al., 2014). The fear memories are stored in the Amygdala (AMY) of the brain. Both the acquisition and extinction of the fear memories occur in the AMY, while the pre-frontal cortex (PFC) controls the expression of fear by the AMY (Sotres-Bayon and Quirk, 2010). This implies that both PFC and AMY are the critical centers and therapeutic intervention in these regions could bring about relief from PTSD symptoms (Koenigs and Grafman, 2009). Selective serotonin reuptake inhibitors are prescribed for the treatment of PTSD. However, these drugs do not produce effective relief from symptoms in more than 50% of the patients and show complete remission only in 20 to 30% of patients (Alexander, 2012). So, there is a requirement for the development of new drugs, which show an effect in all the patients with PTSD (Stein et al., 2000). The disruption of adaptive mechanisms in the brain is found to be due to disruption in the cell signalling pathways involving BDNF, CREB, ERK and caspase (Ross, 2009, Andero and Ressler, 2012). Patients with PTSD were found to have deficiencies in BDNF and CREB (Kim et al., 2017). This removal of

BDNF genes was found to impair spatial memory and loss of aversive memories (Heldt et al., 2007).ERK (extracellular signal-regulated kinases) is another pathway involved in memory and anxiety symptoms. It was found to promote chronic memory and suppress acute adaptive memory (Davis and Laroche, 2006). The apoptotic enzymes like caspase-3 were found to have enhanced activity in the pathology of PTSD (Han et al., 2013). Besides neurotrophic factors, even the levels of the monoamine serotonin are disrupted. There are high levels of serotonin in PTSD patients and drugs with the ability to limit the serotonin activity could be effective in the treatment of PTSD. Serotonin influences mood, aggression, anxiety, sleep, arousal, fear and learning. Hence, the intervention of serotonin could directly influence the symptoms of PTSD (Davis et al., 1997). Further, unlike in stress, there is a disturbance in the HPA functions. There is hypersensitivity of the HPA axis, which leads to a decrease in plasma corticosterone levels. The modulation of the HPA axis could lead to an improvement in stress-related symptoms of PTSD. Few studies have discovered the therapeutic potential of an atypical antipsychotic in the treatment of PTSD in human subjects. Drugs like Olanzapine (OLZ), Aripiprazole (APZ) and Risperidone (RIS) are found to be effective in the treatment of PTSD symptoms in human trials (Petty et al., 2001, Monnelly et al., 2003, Britnell et al., 2017). However, there are no preclinical studies using these atypical antipsychotic drugs to check for their actual mechanisms of action in the treatment of PTSD. These atypicals are reported to possess antiserotonergic and antidopaminergic effects. They do tend to inhibit the activity of both serotonergic and dopaminergic systems. Further, these drugs also have modulating effects on BDNF, CREB, ERK and also caspase enzymes, especially in the PFC and AMY (Reus et al., 2012, Luoni et al., 2014, Rogoz et al., 2017). Hence, we wanted to study the effect of selected atypical antipsychotics in the treatment of PTSD symptoms in an animal model of PTSD in

terms of neurotrophic factors, serotonin and also behavioural parameters like anxiety-like effects and memory deficits.



In the current experiment, we have selected the stress re-stress (SRS) model of PTSD as it is the most appropriate model of PTSD (Liberzon et al., 1997). In this paradigm, animals are subjected to initial traumatic stress and then subsequent "reminder episodes" as contextual triggers for the development of PTSD. This reminder leads to the development of a stable anxiety state and other characteristics similar to PTSD in humans. Clinically, PTSD treatment involves long-term drug administration for a productive outcome. So, an animal model that induces PTSD symptoms chronically would be helpful in drug discovery. With these facts in consideration, a slightly modified version of the SRS model was used for the long-term evaluation of PTSD-related behavioural and physiological changes.



Further, all the selected drugs are atypical antipsychotics are could produce extrapyramidal side effects at higher doses. They also have effects on other stress-related disorders like anxiety, depression and schizophrenia. Hence, before the start of PTSD experimentation, we also evaluated these drugs for their effects on cold restraint stress. We measured the plasma corticosterone, plasma norepinephrine and also brain monoamines to study the effects of atypical antipsychotics under the conditions of stress. All the drugs require prolonged treatment for the beneficial effects to appear. So, all the drugs were given for 21 days in the anti-stress evaluation and 28 days in the anti-PTSD evaluation. The behaviour parameters indicative of the development of PTSD like anxiety and cognitive deficits were evaluated using elevated plus maze (EPM) and Y-maze, respectively.