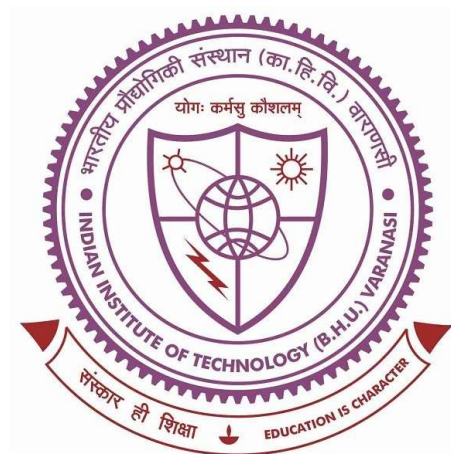


**Pharmacological evaluation of selected atypical antipsychotics
in an experimental model of Post-Traumatic Stress Disorder
(PTSD)**



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

Submitted By

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M. Pharm

Supervisor

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

Dedicated to
my PARENTS



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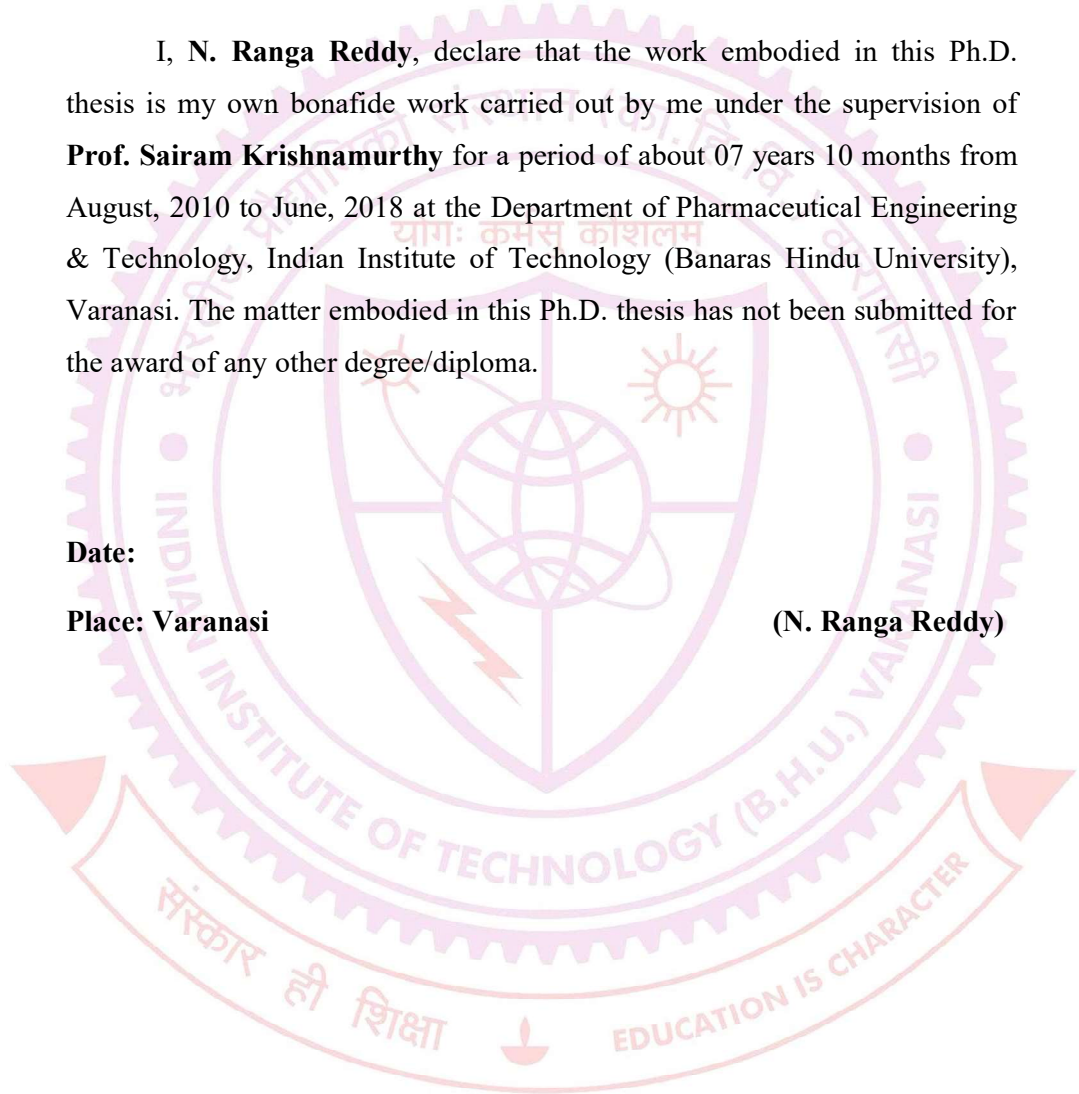
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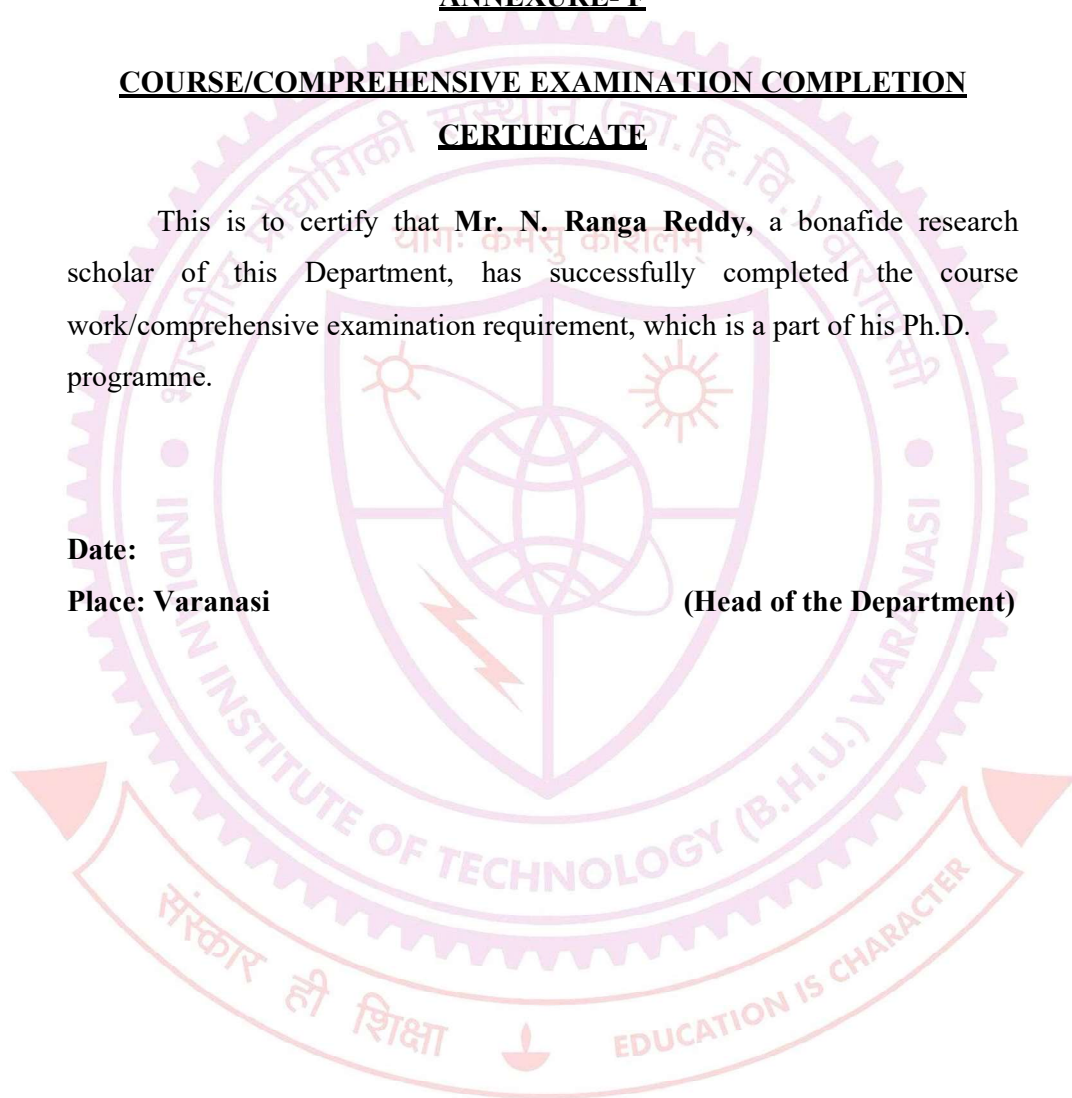
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ANNEXURE- F

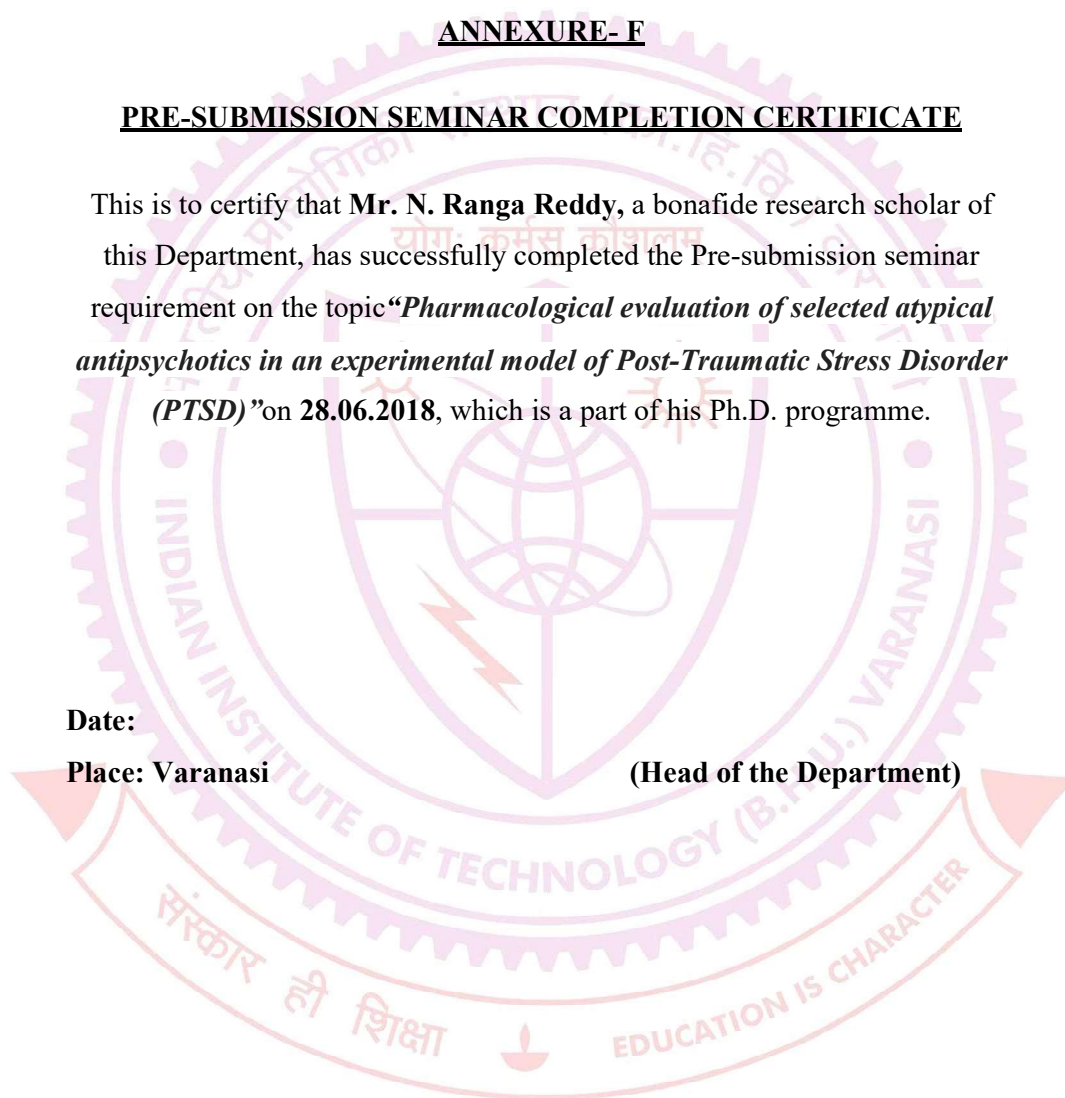
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Acknowledgement

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.

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Table of Contents

Particulars	Page No.
List of Figures	XV
List of Tables	XVII
List of Abbreviations	XVIII
Preface	XX
Introduction	XXI
Chapter 1	
1.1.Introduction	1
1.2. Material and methods	2
1.2.1. Animals	2
1.2.2. Drugs and chemicals	2
1.2.3. Drug treatment	2
1.2.4. Cold Restraint stress (CRS)	2
1.2.5. Evaluation of Catalepsy behaviour	3
1.2.6. Evaluation of Ulcer Index	3
1.2.7. Estimation of plasma corticosterone and norepinephrine by HPLC	4
1.2.8. Estimation of Serotonin, Dopamine and their Metabolites by HPLC	4
1.2.9. Statistics for Data Analysis	4-5
1.3. Results	5
1.3.1. Effect of OLZ on CRS-induced alteration on catalepsy behaviour	5
1.3.2. Effect of OLZ on CRS-induced alteration on ulcer index	6
1.3.3. Effect of OLZ (0.1, 1.0 and 10mg/kg) on CRS-induced alteration on plasma corticosterone	7
1.3.4. Effect of OLZ (0.1, 1.0 and 10mg/kg) on CRS-induced alteration on plasma nor-epinephrine	8
1.3.5. Effect of OLZ (0.1, 1.0 and 10mg/kg) on CRS-induced alteration of serotonin and its metabolite in PFC, HIP and AMY	9-11
1.3.6. Effect of OLZ (0.1, 1.0 and 10mg/kg) on CRS-induced alteration on dopamine level in PFC, HIP and AMY	12
1.4. Discussion	13-14
Chapter 2	
2.1 Introduction	15-16
2.2. Material and methods	17
2.2.1. Animals	17
2.2.2. Drugs and chemicals	17
2.2.3. Drug treatment	17
2.2.4. Cold restraint stress (CRS)	17
2.2.5. Evaluation of Catalepsy behaviour	18

2.2.6. Evaluation of Ulcer Index	18
2.2.7. Estimation of plasma corticosterone and norepinephrine by HPLC	18
2.2.8. Estimation of Serotonin, Dopamine, norepinephrine (NE) and their Metabolites by HPLC	19
2.2.9. Statistics for Data Analysis	20
2.3. Results	21
2.3.1. Effect of aripiprazole (APZ) on CRS-induced alteration in cataleptic behaviour	21
2.3.2. Effect of aripiprazole (APZ) on CRS-induced alteration in ulcer index	21-22
2.3.3. APZ alters plasma CORT and NE in Stressed animal	22-23
2.3.4. APZ selectively alters the level of 5-HT and its metabolites in hippocampus, prefrontal cortex, Amygdala and hypothalamus	24-26
2.3.5. APZ selectively alters level of DA and its metabolite in prefrontal cortex, hippocampus Amygdala and hypothalamus	27-29
2.3.6. Significant Correlation exists between gastric ulcer, plasma corticosterone and monoamines in discrete brain regions	30-31
2.4. Discussion	32-35
Chapter 3	
3.1. Introduction	36-37
3.2. Materials and Methods	37
3.2.1. Animals	37
3.2.2. Drugs	37
3.2.3. Cold Restraint Stress (CRS) model	38
3.2.4. Experimental protocol	38
3.2.5. Evaluation of catalepsy behaviour in bar test	38-39
3.2.6. Estimation of ulcer index	39
3.2.7. Estimation of Plasma Corticosterone (CORT)	39
3.2.8. Estimation of Plasma Norepinephrine (NE)	39-40
3.2.9. Estimation of serotonin, dopamine and their metabolites	41
3.2.10. Statistical analysis	41
3.3. Results	41
3.3.1. Effect of risperidone on catalepsy behaviour in bar test	41
3.3.2. Repeated low dose risperidone decreases Ulcer index due to CRS	42
3.3.3. Risperidone alters plasma CORT and NE in stressed animals	42-43
3.3.4. RIS selectively alters the level of 5-HT and its metabolite in the hippocampus, prefrontal cortex, and striatum	44-45
3.3.5. RIS selectively alters the level of DA and its metabolite in the hippocampus, prefrontal cortex, and striatum	46-48
3.4. Discussion	49-53

Chapter 4	
4.1. Introduction	54
4.2. Materials and methods	55
4.2.1 Drugs & chemicals	55
4.2.2 Animals	55-56
4.2.3 Experimental protocol	56
4.2.4 Stress-restress (SRS)/ Time-dependent sensitization (TDS)	57
4.2.5 Evaluation of freezing-like behaviour	57
4.2.6 Evaluation of anxiety	58
4.2.7 Y-maze	58-59
4.2.8 Estimation of plasma corticosterone by HPLC	59-60
4.2.9 Western blot analysis	60
4.2.9.1 Tissue preparation	60
4.2.9.2 Protein isolation	60
4.2.9.3 Western blotting	60-61
4.2.10 Statistical analysis	61
4.3. Results	62
4.3.1 Effect of OLZ on SRS-induced rise in the Freezing behaviour	62
4.3.2 Effect of OLZ in SRS-induced anxiety behaviour in EPM	63-64
4.3.3 Effect of OLZ on SRS-induced alterations in the spatial memory in Y-maze test	65-66
4.3.4. The effect of OLZ on SRS-induced changes in Y-maze arm discrimination	67-68
4.3.5 Effect of OLZ on the SRS-induced decline in plasma corticosterone	69
4.3.6. Effect of OLZ on the expression of BDNF	69-70
4.3.7. Effect of OLZ treatment on the expression of pERK/ERK in PFC	71
4.3.8. Effect of OLZ treatment on the expression of pERK/ERK in AMY	72
4.3.9. Effect of OLZ on the expression of CREB	73
4.3.10. Effect of OLZ on the expression of Caspase-3	74-75
4.4. Discussion	75-82
Chapter 5	
5.1. Introduction	83-84
5.2. Materials and methods	84
5.2.1. Drugs & chemicals	84
5.2.2. Animals	84
5.2.3. Experimental protocol	85
5.2.4. Stress-restress (SRS)	85-86
5.2.5. Evaluation of freezing	86
5.2.6. Evaluation of anxiety	86
5.2.7. Evaluation of Memory	86-87

5.2.8. Estimation of plasma corticosterone by HPLC	87
5.2.9. Western blot analysis	87-88
5.2.10. Statistical analysis	88
5.3. Results	89
5.3.1. Effect of APZ on open arm entries in EPM	89
5.3.2. Effect of APZ on open arm time spent on EPM	90
5.3.3. Effect of APZ on fecal pellets on EPM	91
5.3.4. Effect of APZ on immobility period	92
5.3.5 Effect of APZ on Y-maze Trail-I	93
5.3.6. Effect of APZ on Y-maze Trail-II	94
5.3.7. Effect of APZ on SRS-induced plasma corticosterone level	95
5.3.8. Effect of APZ on SRS-induced brain serotonin levels	95
5.3.9. Effect of APZ on SRS-induced brain dopamine levels	96
5.3.10. APZ induces the expression of BDNF	97
5.3.11. Effect of APZ treatment on the expression of pERK in PFC	98
5.3.12. Effect of APZ treatment on the expression of pERK in AMY	99
5.3.13. APZ promotes the expression of CREB	100
5.3.14. APZ inhibits the expression of Caspase-3	101
5.4. Discussion	102-103
Chapter 6	
6.1. Introduction	104-106
6.2. Materials and methods	106
6.2.1. Animals	106
6.2.2. Drugs	106
6.2.3. Animal Treatment	107
6.2.4. Modified stress-restress paradigm	107
6.2.5. Evaluation of anxiety-like behaviour	108
6.2.6. Evaluation of Memory	108-109
6.2.7. Estimation of Serotonin and Dopamine by HPLC	109
6.2.8. Western blot analysis	110
6.2.9. Statistical analysis	110-111
6.3. Results	111
6.3.1. RIS Reduced SRS-Induced Anxiety-Like Symptoms in EPM Test	111-112
6.3.2. RIS improved loss of spatial recognition memory in Y-Maze Test	112-113
6.3.3. Effect of RIS (0.1, 1.0 and 10mg/kg) on SRS-induced alteration on 5HT level in PFC and AMY	113-114
6.3.4. Effect of RIS (0.1, 1.0 and 10mg/kg) on SRS-induced alteration on dopamine level in PFC, and AMY	114-115
6.3.5. RIS treatment enhances BDNF formation	115-116
6.3.6. RIS decreases the pERK expression in PFC	117
6.3.7. RIS decreases the pERK expression in AMY	118

6.3.8. RIS enhances the expression of CREB	119-120
6.3.9. RIS mitigates the Caspase-3 expression	121-122
6.4. Discussion	123-126
Summary	127-128
References	129-153
publications from thesis	154

List of Figures

Figure No.	Figure Captions	Page No.
1.1	Effect of OLZ on CRS-induced alteration in catalepsy behaviour	6
1.2	Effect of OLZ on CRS-induced alteration on ulcer index	7
1.3	Effect of OLZ on CRS-induced alteration on plasma-corticosterone	8
1.4	Effect of OLZ on CRS-induced alteration on plasma nor-epinephrine	9
1.5	OLZ alters level of 5-HT	10
1.6	OLZ alters level of 5HIAA	11
1.7	OLZ alters level of 5HIAA/5-HT	12
1.8	Effect of OLZ on CRS-induced alteration on dopamine level	13
2.1	Effect of APZ on CRS-induced cataleptic behaviour	22
2.2	Effect of APZ on CRS-induced ulcer index	23
2.3	APZ alters plasma CORT	24
2.4	APZ alters plasma NE	24
2.5	APZ selectively alters the level of 5-HT	25
2.6	APZ selectively alters the level of 5-HIAA	26
2.7	APZ selectively alters the level of 5-HT/5HIAA	27
2.8	APZ selectively alters the level of dopamine	28
2.9	APZ selectively alters the level of DOPAC	29
2.10	APZ selectively alters the level of DOPAC/DA	30
2.11	Correlation analysis of plasma corticosterone and monoamines	32-33
3.1	Repeated low dose risperidone decreases ulcer index in rats	41
3.2	Effect of risperidone on catalepsy behaviour	42
3.3	Risperidone alters plasma CORT	43
3.4	Risperidone alters plasma Norepinephrine	43
3.5	RIS selectively alters the level of 5-HT	45-46
3.5	RIS selectively alters the level of 5-HIAA	45-46
3.5	RIS selectively alters the level of 5-HIAA/5HT	45-46
3.6	RIS selectively alters the level of DA	48
3.6	RIS selectively alters the level of DOPAC	48
3.6	RIS selectively alters the DOPAC/DA ratio	48
4.1	Schematic representation of the experimental design	56
4.2	Effect of OLZ on the SRS-induced rise in the Freezing behaviour	62
4.3	The effect of OLZ on SRS-induced changes in Y-maze arm	68
4.4	Effect of OLZ on the SRS-induced decline in plasma corticosterone	69
4.5	The effect of OLZ on SRS-induced changes in the expression of BDNF in PFC and AMY	70
4.6	Effect of OLZ treatment on the expression of pERK/ERK in PFC	71

4.7	Effect of OLZ treatment on the expression of pERK/ERK in AMY	72
4.8	Effect of OLZ treatment on the expression of CREB in PFC, AMY	73-74
4.9	Effect of OLZ on the expression of Caspase-3	75
5.1	Schematic diagram of the experimental protocol of APZ in PTSD	86
5.2	Effect of APZ on open arm entries in EPM	90
5.3	Effect of APZ on open arm time spent on EPM	91
5.4	Effect of APZ on fecal pellets on EPM	92
5.5	Effect of APZ on immobility period	93
5.6	Effect of APZ on Y-maze Trail-I	94
5.7	Effect of APZ on Y-maze Trail-II	95
5.8	Effect of APZ on SRS-induced plasma corticosterone level	96
5.9	APZ induces the expression of BDNF	98
5.10	Effect of APZ treatment on the expression of pERK in PFC	99
5.11	Effect of APZ treatment on the expression of pERK in AMY	100
5.12	APZ promotes the expression of CREB	101
5.13	APZ inhibits the expression of Caspase-3	102
6.1	RIS treatment enhances BDNF formation	118
6.2	RIS decreases the pERK expression in PFC	119
6.3	RIS decreases the pERK expression in AMY	120
6.4	RIS enhances the expression of CREB	121
6.5	RIS mitigates the Caspase-3 expression	123

List of Tables

Table No.	Table Captions	Page No.
4.1	Effect of OLZ and PAX in the open arm	64
4.2	Effect of OLZ and PAX in trial-1 and trial-2	66
5.1	APZ Effect on 5HT in SRS rats brain regions	96
5.2	APZ effect on DA in SRS rat brain regions	96
6.1	RIS Reduced SRS-Induced Anxiety-Like Symptoms in EPM Test	111-112
6.2	RIS improved SRS-induced loss in spatial recognition memory in Y-maze test	114
6.3	RIS effect on 5HT in SRS rats	114
6.4	RIS effect on DA in SRS-induced rat brain	115

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
\leq	:	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.

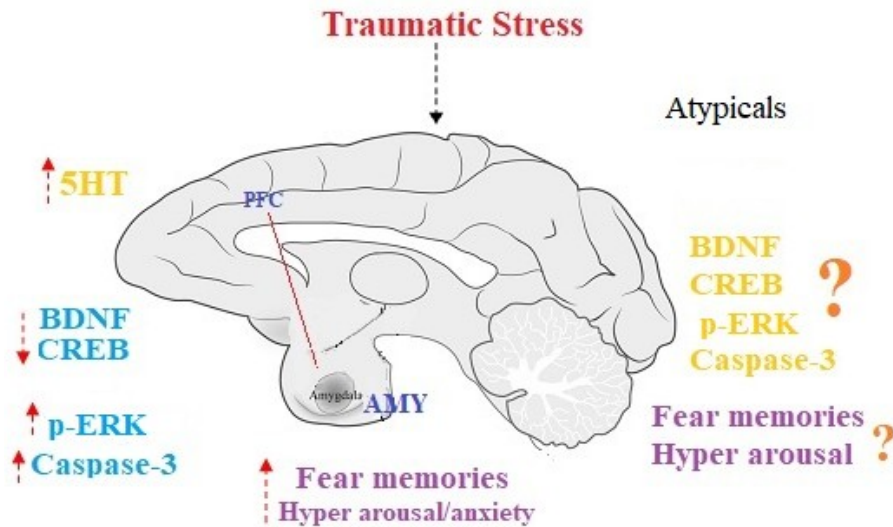
Further, the effect on serotonin was also estimated. **Chapter 5** describes the anti-PTSD potential of aripiprazole in the stress-restress model of rats with an emphasis on plasma corticosterone and neurotrophic factors. **Chapter 6** evaluated the effect of risperidone in the stress re-stress model 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

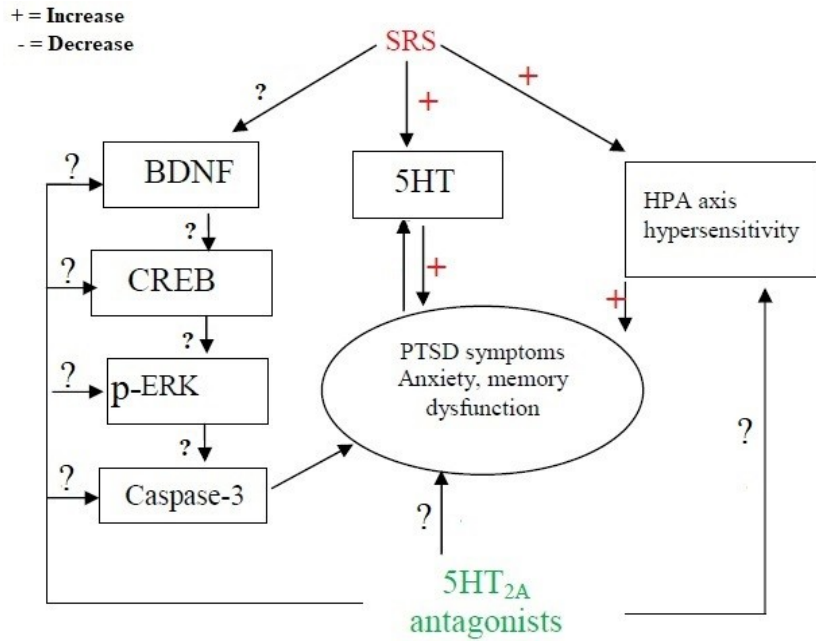
Post-traumatic stress disorder (PTSD) is one of the anxiety disorders that occurs in people who have undergone terrifying experiences. These experiences are traumatic and the symptoms can last for prolonged periods. As per NIH, PTSD occurs due to experiences like the death of loved ones, sexual assault, 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 and 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.