Evaluation of anti-PTSD like effect of olanzapine against stress re-stress model in rats

4.1 Introduction

Olanzapine is an atypical anti-psychotic with potent 5HT2 antagonism. At a higher dose, it also blocks D2 receptors. There are only limited data regarding the clinical therapeutic potential of atypical antipsychotics for the treatment of PTSD symptoms (Adetunji *et al.*, 2005). In particular, OLZ, clozapine, quetiapine and risperidone were found to be beneficial in the amelioration of PTSD symptoms (Hamner, 1996; Monnelly et al., 2003; Petty et al., 2001; Sokolski et al., 2003). However, the preclinical data on the pharmacological effects of OLZ in animal models of PTSD are lacking. Preclinical studies would help in better understanding the underlying mechanisms of drugs and further determine the pharmacological claims. The prefrontal cortex (PFC) and amygdala (AMY) are the critical brain centres responsible for the development and pathophysiology of PTSD (Koenigs *et al.*, 2009). AMY is responsible for the acquisition and extinction of fear memories (Pape *et al.*, 2010), while the PFC has a stringent control on the expression of fear (Sotres-Bayon *et al.*, 2010). Therapeutic interventions that target these two brain centers could offer effective therapy for PTSD (Koenigs *et al.*, 2009). The chronic occurrence of PTSD symptoms is mainly due to the interruption of the adaptive mechanisms in the brain like BDNF, ERK, CREB and caspase signalling pathways (Andero *et al.*, 2012; Ross, 2009). Previous studies have found that combat war veterans with PTSD had disturbances in BDNF gene transcription and the CREB pathway (Kim *et al.*, 2017; Martini *et al.*, 2013). BDNF gene knockout mice demonstrated impaired spatial memory and loss of aversive memories (Heldt *et al.*, 2007). Further, increased levels of phosphorylated ERK are thought to play a critical role in the pathophysiology of PTSD in rats (Whitaker *et al.*, 2016). ERK (extracellular signal-regulated kinases) pathway promotes chronic memory and suppresses acute adaptive

memory (Davis *et al.*, 2006). It has been demonstrated that OLZ offers neuroprotection by stimulating the expression of BDNF and CREB proteins in the brain (Lee *et al.*, 2010; Park *et al.*, 2013; Réus *et al.*, 2012). Moreover, OLZ also inhibits apoptotic cell death in the brain by decreasing caspase-3 activation (Wang *et al.*, 2005).

So, with this informational background, we have hypothesized and evaluated the effects of OLZ on rats subjected to the PTSD model with specific emphasis on neurotrophic cell signalling pathways involving BDNF, CREB and ERK and also cell destructive pathways involving caspase. Further, we have evaluated the plasma corticosterone and behavioural parameters like freezing, memory and anxiety as the hallmark symptoms of PTSD.

4.2 Materials and methods

4.2.1 Drugs & chemicals

OLZ and PAX were provided as gift samples by Ranbaxy Laboratories, India. Both of them were formulated into suspensions in a solution of 0.5% carboxymethylcellulose (CMC) in water. Antibodies used for the western blot studies were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, California, USA). All the other chemicals and reagents of analytical grade were procured from local suppliers.

4.2.2 Animals

Adult male Charles Foster albino rats weighing between 220-260 g were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University. They were housed in polypropylene cages at an ambient temperature of 25±1^{o}C; and 45–55% Relative Humidity (RH). Food and water were provided *ad libitum*. Approval for the experimentation was obtained from the Institutional animal ethical committee (Ref No. Dean/10-11/148). All the experiments were performed following the principles of laboratory animal care (National Research Council US Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011) guidelines. Every possible effort was taken to minimize the

suffering and the number of animals used. All the experiments were conducted between 08:00 and 16:00hrs.

4.2.3 Experimental protocol

The experimental protocol was carried for 29 days, as depicted in Fig-4.1. Animals were randomly assigned into six groups with 6 (n=6) in each as control (group-1), PTSD control (group-2), OLZ doses as 0.1mg/kg, 1.0 mg/kg and 10 mg/kg (group-3, 4, and 5 respectively) and PAX-10mg/kg (group-6). Before the start of the experiment, rats were subjected to a baseline test session of behavioural paradigms like freezing, anxiety and memory. After the day of initial stress exposure, rats were given a single dose of treatment daily for the next 28 days. Group 1 and 2 received 0.5% CMC suspension. Groups 3, 4 and 5 received orally 0.1, 1.0 and 10mg/kg doses of OLZ suspensions. Group 6 received a PAX dose of 10mg/kg through the same oral route. Behavioural assessments for freezing, anxiety and memory were conducted on day-1, 7, 14, 21 and 28th day of the drug treatment schedule. Following the 2hr of restress procedure, the animals were subjected to freezing analysis, elevated plus maze (EPM) test and the Y-maze test with a lag of 5 min between each consecutive test. On the last day, animals were decapitated; the blood was collected from trunk and their brain regions like PFC and AMY were isolated and stored at -80^{0}C until further processing.

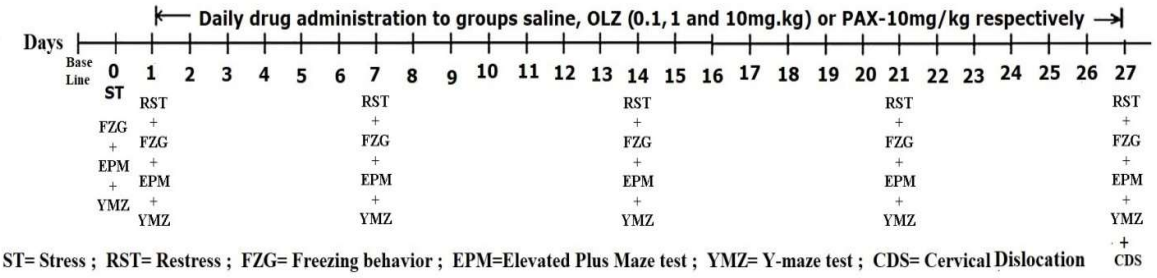

Fig-4.1: The schematic representation of the experimental design. Here '+' indicates performed.

4.2.4 Stress-restress (SRS)/ Time-dependent sensitization (TDS)

SRS model was used to induce PTSD in rats wherein they are exposed to prolonged variable stress and then time-dependent stress sensitization paradigm (Krishnamurthy *et al.*, 2013; Liberzon *et al.*, 1997). Initially, animals were subjected to a single session of prolonged stress for 2hrs in a metallic restrainer on Day-0. Immediately they were subjected to 20 min forced swim test in an 18cm swim tank at an ambient temperature of 25^0 C. They were allowed to recover for 15min and then promptly exposed to 0.8ml of 4% halothane vapors (stress) until a brief loss of consciousness. After recuperation from anaesthesia, they were returned to their home cages. Consequently, starting from D-1, these animals were re-exposed to 20 min forced swim stress (restress) on D-7, 14, 21 and D-28 to enhance the sensitization (Liberzon *et al.*, 1997).

4.2.5 Evaluation of freezing-like behaviour

Freezing-like behaviour was evaluated in all the animals by exposing them to the reminder situation for 5 min on days 1, 7, 14, 21 and 28^{th} day, respectively. With modification in the experiment, once the animals were removed from the forced swim test, they were placed on an elevated table, which is of equivalent height to the cylinder standing just beside it. The animals showed different behavioural patterns like rearing, grooming and freezing-like behaviour (absence of all movements except for breathing). The freezing behaviour was measured over a period of 5min using a video tracking system (Kondaurova et al., 2015; Krishnamurthy et al., 2013). The total cumulative time maintained by the animal in freezing posture was measured and scored by ANY-MAZE^{TM} video tracking software.

4.2.6 Evaluation of anxiety

Animals were evaluated for anxiety-like behaviour using EPM on days 1, 7, 14, 21, and 28th after restress procedure in PTSD-induced rats (Walf et al., 2007). The aversion to heights and open spaces is found to be the cause of anxiety-like behaviour in rats when exposed to the EPM (Carobrez et al., 2005). The fabricated maze has two opposite arms, 50×10 cm, crossed with enclosed arms of the same dimension but having a 40 cm high wall. The arms were connected to a central square, 10×10 cm, giving the apparatus shape of a plus sign. The maze was kept in a dimly lit room and elevated 50 cm above the floor. Naive rats were placed individually in the center of the maze, facing a closed arm. After that number of entries and time spent on both the open and enclosed arm were recorded during the next 5 minutes using the ANY-MAZE^{TM} video tracking system. An arm entry was defined when all four paws of the rat were in the arm. The behavioural parameters were assessed by a person unaware of the experimental protocol.

4.2.7 Y-maze

The behaviours on Y-maze were evaluated on all the days of restress procedure. Y maze is specifically used to assess spatial recognition memory (Dellu et al., 1992). The novelty-seeking tendency of rats is exploited to check the memory traits using Y-maze (Wright et al., 2005). The maze consists of three identical arms starting from a central point (50 cm long, 16 cm wide and 32 cm high) with 120^{o} angles to each other. The floor of the apparatus was covered with soiled animal bedding. Colored papers were fixed around the perimeter of the maze as visual cues, and they were changed for each test to maintain novelty to the animals. The arms were designated as starting arm, familiar arm, and a novel arm. In the first trial, entry to the novel arm was blocked, and rats were allowed to move within the other two arms for 15 minutes. Four hours after the first trial, animals were allowed to access all three arms for 5 min, and the numbers of entries were recorded. An arm entry was counted when the

head and two front paws were inside the arm. The entire recordings were done using ANY-MAZE^{TM} video tracking software. The behavioural patterns like the total number of entries into all the arms (for the 5 min of trial I and the percentage entries in known and novel arms for the 5 min period of trial two were measured. The total number of entries into all the arms (for the 5 min of trial I and II) is indicative of general exploration attitude (curiosity) and the percentage entries in known versus novel arm for the 5 min period of trial two is as a measure of arm discrimination (spatial recognition memory). The percentage of time spent in the novel arm to time spent in all arms and in the center of the apparatus during trial II is determined as the coping behaviour to the novel arm. Any decrease in the coping behaviour in the novel arm is indicative of the increase in anxiety-like behaviour (Poimenova et al., 2010).

4.2.8 Estimation of plasma corticosterone by HPLC

Approximately 2mL of the blood sample was collected from the rat's trunk immediately after decapitation. The blood was subjected to centrifugation in heparinized tubes to separate the plasma. The plasma corticosterone was measured by using High-performance liquid chromatography (HPLC) connected with a UV detector (Waters, USA) (Krishnamurthy et al., 2011; Woodward et al., 1987). An isocratic mobile phase consisting of a mixture of methanol: water in the ratio of 70:30 was run through an HPLC column (Waters: Spherisorb, RP C18, 5-µm particle size, 4.6 mm i.d. and 250 mm at 30°C). A 500 µL of plasma containing a known quantity of dexamethasone (as internal standard) was extracted with 5mL of dichloromethane. This dichloromethane extract was evaporated to dryness and then dissolved in 100 µL of the mobile phase. Twenty microliters of the extract were injected into the HPLC system for quantification at a flow rate of 1.2 mL/min. The corticosterone was detected at 250nm using a UV detector (Model 2849, Waters, USA). The chromatogram was

recorded and analyzed with Empower software.

4.2.9 Western blot analysis

4.2.9.1 Tissue preparation

Rats were decapitated and their skull was cut along the coronal suture and sagittal suture. The PFC and AMY were collected bilaterally and immersed immediately in liquid nitrogen and stored at -80° C for further protein isolation.

4.2.9.2 Protein isolation

The brain tissue was homogenized in a lysis buffer supplemented with a protease inhibitor cocktail. This tissue sample was then centrifuged at 14000g at 4°C for 30min and the resultant protein-containing supernatant was stored at -80°C. The protein concentration was determined by using a standard plot of bovine serum albumin (Bradford, 1976).

4.2.9.3 Western blotting

A standard plot was generated using bovine serum albumin. An aliquot of each sample was electrophoresed in 10% SDS-PAGE gels for BDNF, pERK, ERK, CREB and caspase-3 proteins, transferred to polyvinylidene fluoride membranes and probed with specific antibodies. The membrane was incubated overnight with rabbit ERK 1/2 (1:500, 41 kDa; ab196883; Abcam plc., India), rabbit pERK (1:500, 44 kDa; ab214362; Abcam plc., India), sheep anti-BDNF (1:500, 28 kDa; ab24491; Abcam plc., India), rabbit CREB (1:500, 43 kDa; ab5803; Abcam plc., India) and rabbit active anti-caspase-3 (1:1000, 32 kDa; ab90437; Abcam plc., India) polyclonal primary antibodies. After the detection of the desired antibodies against the proteins of interest, the membrane was stripped with stripping buffer (25 mM Glycine pH 2.0, 2% SDS) for 30 min at room temperature. It was re-probed overnight with rabbit anti-β-actin (1:500, 42 kDa; ab93027; Abcam plc., India) polyclonal primary antibody to confirm equal loading of protein. Further, the membrane was probed

with secondary antibodies, anti-rabbit Cy5 (1:200, ab97051; Abcam plc., India). An immunoreactive band of proteins was detected by chemiluminescence detector (Fusion FX vilber lourmat) using enhanced chemiluminescence (ECL) reagents (Amersham Bioscience, USA). The densitometric scan of films was performed for the quantification of results. The immunoreactive area was determined by densitometric analysis using Biovis gel documentation software.

4.2.10 Statistical analysis

The results obtained were analyzed statistically using GraphPad Prism version-5 software. Behavioural data of the freezing activity, EPM, the total entries and the arm discrimination behaviour between known and novel arm in Y-maze was measured by using repeated measures two-way analysis of variance (ANOVA) with Bonferroni post hoc test. The data of plasma corticosterone and western blot were analyzed with one-way ANOVA with Newman-keuls post hoc test. All the data are represented as the mean ± standard error of the mean (S.E.M). $p < 0.05$ was considered statistically significant.

4.3 Results

4.3.1 Effect of OLZ on SRS-induced changes in the Freezing behaviour

Fig-4.2 shows the effect of repeated treatment of OLZ (0.1, 1.0 and 10mg/kg) and PAX-10.0 mg/kg on SRS-induced changes in freezing behaviour. Statistical analysis by repeated measures two way ANOVA revealed that there was a significant difference among groups [F (5, 180) - 139.4, $p<0.05$], time [F (5, 180) - 137.3, $p<0.05$] and interaction between group and time [F (25, 180) - 26.54, $p<0.05$]. Post-hoc analysis showed that there was no change in freezing behaviour among groups on trial day and D-1. SRS significantly increased the freezing behaviour from D-7 up to D-28 compared to control.

Repeated treatment with OLZ at doses of 1.0 and 10mg/kg significantly alleviated the SRS-induced enhancement in freezing behaviour on D-21 and D-28. However, PAX-10mg/kg showed a significant decline in the freezing behaviour from D-14 and was maintained until D-28. There was no significant difference between OLX (1&10mg/kg) and PAX-10mg/kg on D-28.

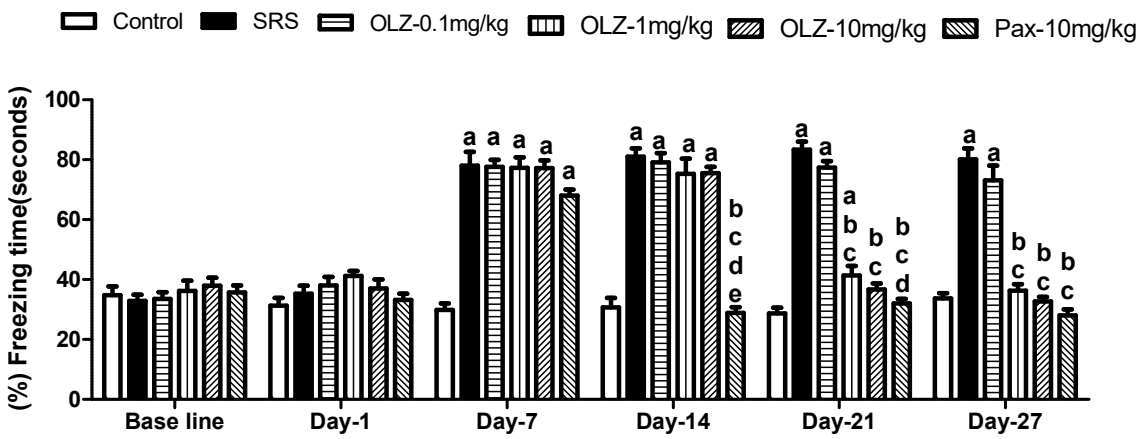

Fig-4.2: All the values are Mean±SEM (n = 6). [a]P < 0.05 compared to control, [b]P< 0.05 compared to SRS control (SRS), [c]P < 0.05 compared to OLZ (0.1 mg/kg), [d]P < 0.05 compared to OLZ (1 mg/kg) and [e]P < 0.05 compared to OLZ (10mg/kg). [Repeated measure two-way ANOVA followed by Bonferroni test].

4.3.2 Effect of OLZ in SRS-induced anxiety behaviour in EPM

The effect of repeated treatment with OLZ (0.1, 1, 10mg/kg) and PAX (10mg/kg) on changes induced by SRS in open arm entries, time spent, the number of fecal pellets, immobility period and the number of total arm entries in EPM were depicted in **Table-1**. Analysis by repeated-measures two-way ANOVA showed that there were significant differences in percentage in open arm entries and time spent, number of fecal pellets and immobility period among groups ([F (5, 180) – 77.56; $P < 0.05$], [F (5, 180) - 30.49; $P < 0.05$], [F (5,180) - 57.29; $P < 0.05$], [F (5,180) - 95.26; $P < 0.05$] respectively), time ([F (5, 180) - 139.8; $P < 0.05$], [F (5, 180) - 29.88; $P < 0.05$], [F (5,180) - 59.37; $P < 0.05$], [F (5, 180) - 182.3; $P < 0.05$] respectively) and an interaction between group and time ([F (25, 180) – 10.76; $P < 0.05$], [F (25,180) - 4.190; $P < 0.05$], [F (25,180)- 10.11; $P < 0.05$], [F (25, 180) - 19.94; $P < 0.05$], respectively) in the EPM paradigm. However, no significant differences were observed in the number of total entries among groups [F (5, 180) - 1.956; $P > 0.05$], time [F (5, 180) - 1.028; $P > 0.05$], and there was no significant interaction between group and time [F (25, 180) - 1.048; $P > 0.05$]. Post-hoc analysis revealed a significant difference among groups in the percentage of open arm entries and time spent, the number of fecal pellets and immobility. The percentage of open arm entries and time spent were decreased due to stress from D-7 to D-28 compared to control rats. Stress also increased the number of fecal pellets and immobility period compared to control rats. Treatment with OLZ 10mg/kg significantly enhanced the SRS-induced decrease in the percentage of open arm entries and time spent on D-21 and D-28. Further, OLZ 10mg/kg significantly reduced the SRS instigated a rise in the number of fecal pellet droppings and immobility period on D-21 and D-28. PAX (10mg/kg) treatment also alleviated the SRS-induced decline in the percentage of open arm entries, time spent and increase in fecal droppings and immobility time on D-1and this effect continued up to D-28. There was no significant difference between OLZ 10mg/kg and PAX on D-28 on all the paradigms of EPM. However, OLZ-1mg/kg did not show any changes regarding open

arm entries and time spent on all the days tested. However, it showed a significant reduction in fecal droppings and immobility periods compared to the SRS group on D-21 and D-28.

Table 4.1: Effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS exposed rats in terms of percentage number of entries and time spent in open arm, fecal pellets, immobility time and total arm entries.

Day	Control	SRS	OLZ-0.1	OLZ-1	OLZ-10	PAX
Open arm entries (in percent)						
Base line	30.12 ± 1.790	25.40 ± 1.30	27.67 ± 1.12	28.51 ± 1.78	25.91 ± 1.450	29.70 ± 1.11
1	27.0 ± 1.10	26.80 ± 2.30	28.60 ± 1.26	27.82 ± 2.21	26.65 ± 1.17	27.70 ± 0.97
7	28.0 ± 1.34	07.30 ± 1.07[a]	08.31 ± 0.53[a]	09.10 ± 0.70[a]	10.04 ± 0.20[a]	9.2 0 ± 0.67[a]
14	27.4 ± 1.50	10.60 ± 0.90[a]	11.20 ± 0.80[a]	12.07 ± 0.89[a]	13.80 ± 0.46[a]	22.80 ± 1.96[bcde]
21	31.1 ± 1.31	14.22 ± 0.73[a]	15.02 ± 1.01[a]	17.54 ± 1.90[a]	27.92 ± 0.75[bcd]	29.04 ± 1.10[bcd]
28	30.46 ± 1.28	15.40 ± 0.61[a]	16.87 ± 1.03[a]	18.30 ± 2.23[a]	28.30 ± 1.50[bcd]	28.40 ± 1.01[bcd]
Open arm time spent (in percent)						
Base line	9.21 ± 0.78	8.31 ± 1.01	8.60 ± 0.57	9.01 ± 0.86	8.21 ± 0.830	8.31 ± 0.59
1	8.13 ± 0.41	7.81 ± 0.38	7.95 ± 0.66	8.18 ± 0.51	7.95 ± 0.720	8.50 ± 0.67
7	9.4 ± 0.52	3.20 ± 0.41[a]	3.92 ± 0.18[a]	3.20 ± 0.70[a]	3.89 ± 0.620[a]	4.36 ± 0.70[a]
14	8.7 ± 0.74	4.20 ± 0.46[a]	4.30 ± 0.43[a]	4.10 ± 0.53[a]	4.84 ± 0.310[a]	7.10 ± 0.50[bcd]
21	9.7 ± 0.56	3.60 ± 0.63[a]	4.00 ± 0.82[a]	4.84 ± 0.37[a]	8.00 ± 1.010[bcd]	9.0 ± 0.48[bcd]
28	9.3 ± 1.30	3.50 ± 0.70[a]	5.00 ± 1.00[a]	5.29 ± 0.50[a]	8.40 ± 0.640[bcd]	9.23 ± 0.38[bcd]
Fecal pellets (in numbers)						
Base line	3.94 ± 0.450	4.41 ± 0.39	4.12 ± 0.68	3.99 ± 0.42	3.67 ± 0.460	4.10 ± 0.54
1	4.02 ± 0.22	4.50 ± 0.30	4.48 ± 0.72	4.48 ± 0.31	4.13 ± 0.620	4.36 ± 0.70
7	3.67 ± 0.40	6.10 ± 0.64[a]	5.90 ± 0.53[a]	6.30 ± 0.41[a]	6.10 ± 0.430[a]	6.05 ± 0.67[a]
14	3.80 ± 0.34	5.80 ± 0.71[a]	6.30 ± 0.46[a]	5.90 ± 0.50[a]	5.80 ± 0.290[a]	3.90 ± 0.84[bcde]
21	3.64 ± 0.70	5.59 ± 0.43[a]	5.60 ± 0.31[a]	4.40 ± 0.96[bc]	4.02 ± 0.370[bc]	3.30 ± 0.47[bcd]
28	3.40 ± 0.24	6.06 ± 0.37[a]	5.40 ± 0.28[a]	3.60 ± 0.39[bc]	3.53 ± 0.260[bc]	3.43 ± 0.35[bc]
Immobility (in seconds)						
Base line	9.71 ± 0.460	9.5 ± 0.89	10.1 ± 0.84	10.2 ± 1.49	9.93 ± 1.030	10.4 ± 0.77
1	9.5 ± 0.30	9.4 ± 0.20	9.7 ± 0.24[a]	9.1 ± 0.31	9.0 ± 0.272	9.5 ± 0.37
7	10.4 ± 0.54	34.7 ± 1.70[a]	36.0 ± 2.86[a]	37.0 ± 3.09[a]	38.0 ± 1.302[a]	37.5 ± 1.78[a]
14	8.90 ± 0.34	36.0 ± 2.92[a]	35.6 ± 3.04[a]	34.0 ± 2.21[a]	32.0 ± 1.472[a]	13.0 ± 1.24[bcde]
21	11.3 ± 1.74	37.0 ± 3.52[a]	34.6 ± 1.39[a]	25.2 ± 2.01[abc]	15.0 ± 1.720[bcd]	12.0 ± 1.33[bcd]
28	10.6 ± 0.45	35.0 ± 2.36[a]	35.9 ± 1.27[a]	16.2 ± 1.92[bc]	14.0 ± 1.192[bc]	12.7 ± 1.07[bc]
Total arm entries (in numbers)						
Base line	7.78 ± 0.67	9.01 ± 1.10	9.00 ± 0.780	8.9 ± 0.820	8.7 ± 0.900	9.1 ± 0.850
1	8.10 ± 0.88	8.9 ± 0.90	9.15 ± 0.80	9.5 ± 1.23	7.9 ± 0.672	8.5 ± 1.37
7	7.97 ± 1.01	9.3 ± 0.93	8.60 ± 0.84	9.2 ± 0.77	8.9 ± 1.272	9.1 ± 0.88
14	8.70 ± 0.73	8.3 ± 0.80	9.12 ± 0.82	8.7 ± 0.39	9.4 ± 0.572	9.4 ± 0.70
21	9.50 ± 0.90	9.9 ± 1.79	9.20 ± 2.21	8.7 ± 1.31	9.2 ± 0.720	8.8 ± 1.37
28	8.20 ± 1.30	8.9 ± 1.54	8.69 ± 1.32	9.7 ± 1.80	8.9 ± 1.720	8.4 ± 1.50

All the values represent Mean±SEM (n = 6). Repeated measures two-way ANOVA analysis with Bonferroni Post-hoc test. [a]$P < 0.05$ compared to control, [b]$P < 0.05$ compared to SRS control (SRS), [c]$P < 0.05$ compared to OLZ (0.1 mg/kg), [d]$P < 0.05$ compared to OLZ (1 mg/kg) and [e]$P < 0.05$ compared to OLZ (10mg/kg).

4.3.3 Effect of OLZ on SRS-induced alterations in the spatial memory in Y-maze test

The effect of repeated OLZ (0. 1, 1.0 and 10mg/kg) and PAX-10mg/kg treatment on SRS-induced changes in exploratory behaviour (curiosity) in trial-1 and trial-2 are presented in **Table-2**. Analysis with repeated measure two-way ANOVA showed significant differences of curiosity in both trial-1 and trial-2 among groups ([F (5, 180) - 90.51; $P < 0.05$] and [F (5, 180) - 19.8 16.053; $P < 0.05$] respectively), time ([F (5,180) - 161.7; $P < 0.05$] and [F (5, 180) - 32.54; $P < 0.05$] respectively) and an interaction between group and time ([F (25, 180) - 21.82; $P < 0.05$] and [F (25, 180) - 3.742; $P < 0.05$] respectively). Further, Post-hoc analysis revealed that the stress paradigm showed a significant decrease in curiosity behaviour in comparison to the control rats from D-7 to D-28. OLZ in the doses of 1 and 10mg/kg significantly mitigated the SRS-induced decrease in curiosity behaviour on D-21 & D-28. However, the treatment with PAX-10mg/kg reversed this SRS-induced decrease in curiosity on D-14, D-21, and D-28 persistently. Also, no significant differences were observed between OLZ (1 and 10mg/kg) and PAX on D-28 regarding curiosity behaviour in the Y-maze paradigm. The data of the percentage of time spent (coping behaviour) in the novel arm is presented in table-2. Repeated measures two-way ANOVA showed significant differences of percentage of time spent in the novel arm among groups ([F (5, 180)- 13.65; $P < 0.05$], time ([F (5,180) - 14.84; $P < 0.05$] and an interaction between group and time [F (25, 180) - 2.537; $P < 0.05$] respectively). SRS significantly decreased the percentage of time spent in the novel arm compared to control from D-7 till D-28. Repeated treatment with OLZ-1 and 10mg/kg showed a significant increase in the time spent in the novel arm that was decreased by SRS from D-21 to D-28. PAX-10mg/kg showed the reversal in an SRS-induced decrease of novel arm time spent from D-14 till D-28. Also, there were no significant differences in effects in percentage time spent in the novel arm in between OLZ (1 and 10mg/kg) and PAX-10mg/kg on D-21 and D-28.

Table 4.2: Effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS exposed rats in terms of changes in total arm entries during trial-1 and trial-2, indicating curiosity behaviour.

Day	Control	SRS	OLZ-0.1	OLZ-1	OLZ-10	PAX
Total arm entries trial-I (in numbers)						
Base line	7.1 ± 0.73	7.5 ± 075	7.4 ± 0.62	7.33 ± 0.54	7.20 ± 0.55	7.3 ± 0.57
1	7.0 ± 0.62	7.7 ± 1.01	7.6 ± 0.54	7.50 ± 0.43	7.3 ± 0.58	7.7 ± 0.86
7	7.4 ± 1.10	3.0 ± 0.30 [a]	3.0 ± 0.26[a]	3.50 ± 0.21[a]	3.9 ± 0.97 [a]	3.8 ± 0.47 [a]
14	7.3 ± 0.81	3.6 ± 0.73 [a]	4.0 ± 0.66[a]	3.80 ± 0.34 [a]	4.1 ± 0.62 [a]	6.9 ± 0.59 [bcde]
21	7.2 ± 0.41	3.2 ± 0.46 [a]	3.7 ± 0.37 [a]	6.70 ± 0.53[bc]	7.1± 0.52[bc]	7.1 ± 0.72 [bc]
28	7.6 ± 0.21	3.8 ± 0.55 [a]	3.6 ± 0.42 [a]	7.09 ± 0.77[bc]	7.27±0.47[bc]	7.5 ± 0.69 [bc]
Total arm entries trial-II (in numbers)						
Base line	14.2± 0.84	14.7 ± 1.04	15.1 ± 0.98	14.98 ± 0.79	14.56± 1.20	15.2 ± 0.85
1	14.0 ± 0.70	15.0 ±1.10	14.5 ± 0.60	15.80 ± 0.91	14.8 ± 1.50	15.80 ± 1.31
7	15.4± 0.76	7.9 ± 0.70 [a]	9.0 ± 0.90[a]	8.30 ± 0.79 [a]	7.9± 0.90[a]	8.02 ± 0.67 [a]
14	15.6± 0.91	8.3 ± 1.60 [a]	8.7 ± 1.80[a]	8.80 ± 0.82[a]	9.1 ± 0.57 [a]	12.70 ± 0.45[bcd]
21	14.7 ± 0.60	8.1 ± 0.34 [a]	9.5 ± 0.8[a]	11.76± 0.61[b]	13.0± 0.54[b]	13.50 ± 0.76[bc]
28	15.2 ± 1.01	8.4 ± 0.93 [a]	10.0± 0.76[a]	13.90±1.50[bc]	14.2±1.21[bc]	14.60 ± 0.99 [bc]
[(Time spent in Novel arm/(time spent in all the arms and center))*100]						
Base line	25.12± 1.93	24.45± 1.69	24.58± 0.97	26.02± 1.16	25.30 ± 1.45	24.88 ± 1.24
1	24.61±1.68	25.90±1.64	25.48±1.10	26.78±1.26	25.80±2.11	26.60±1.80
7	26.70±1.13	17.89±0.62[a]	17.71±1.09[a]	17.96±1.10[a]	18.70±1.80[a]	17.90±2.41[a]
14	27.90±2.21	15.95±1.56[a]	18.60±1.91[a]	19.95±1.63[a]	19.63±2.04[a]	25.70±1.22[bcde]
21	26.04±1.76	17.14±1.86[a]	19.12±1.56[a]	22.76±0.97[b]	22.90±1.28[b]	24.98±1.86[bc]
28	25.33±1.24	16.40±0.85[a]	19.43±1.34[a]	24.48±1.05[b]	24.67±1.11[b]	25.14±1.58[bc]

All the values represent Mean±SEM (n = 6). Repeated measures two-way ANOVA analysis with Bonferroni Post-hoc test. [a]$P < 0.05$ compared to control, [b]$P < 0.05$ compared to SRS control (SRS), [c]$P < 0.05$ compared to OLZ (0.1 mg/kg), [d]$P < 0.05$ compared to OLZ (1 mg/kg) and [e]$P < 0.05$ compared to OLZ (10mg/kg).

4.3.4. The effect of OLZ on SRS-induced changes in Y-maze arm discrimination

Fig.4.3 depicts the effect of OLZ (0.1, 1 and 10 mg/kg) and PAX-10mg/ kg on SRS-induced changes in spatial recognition memory on D-1, D-7, D-14, D-21, and D-28. Fig-4.3A depicts the entry of rats into the known arm and Fig-3B depicts entry into the Novel arm. Two-way ANOVA of data of Fig-4.3A showed significant differences among groups [$F (5, 180) - 37.15$, $p<0.05$], time [$F (5, 180) - 34.22$, $p<0.05$] and interaction between group and time [$F (25, 180) - 7.264$, $p<0.05$]. Post-hoc analysis of the data showed that there were no changes in entries into the known arm on trial day and D-1. However, SRS significantly increased the entries into known arms from D-7 up to D-28 compared to control. OLZ at doses of 1 and 10mg/kg significantly decreased the entries into the known arm on D-28. PAX-10 significantly alleviates the SRS-induced increase in entries into the known arm from D-14 to D-28. Further, there was no significant difference of entries into known arm between doses of OLZ-1 and 10mg/kg and PAX-10mg/kg on D-28. Two-way ANOVA analysis of data in Novel arm entries is shown in Fig-4.3B showed significant differences among groups [$F (5, 180) - 37.17$, $p<0.05$], time [$F (5, 180) - 50.65$, $p<0.05$] and interaction between group and time [$F (25, 180) - 5.810$, $p<0.05$]. On analysis of data by post-hoc analysis, there were no variations in entries into the Novel arm on D-1. However, SRS significantly decreased the entries into the Novel arm from D-7 up to D-28 in comparison to control. OLZ at doses of 1 and 10mg/kg significantly enhanced the entries into the known arm on D-28. On the other hand, PAX significantly increased the entries into a known arm from D-14 to D-28. Further, there were no significant differences of entries into the Novel arm on D-28 between OLZ (1 and 10mg/kg) and PAX-10mg/kg.

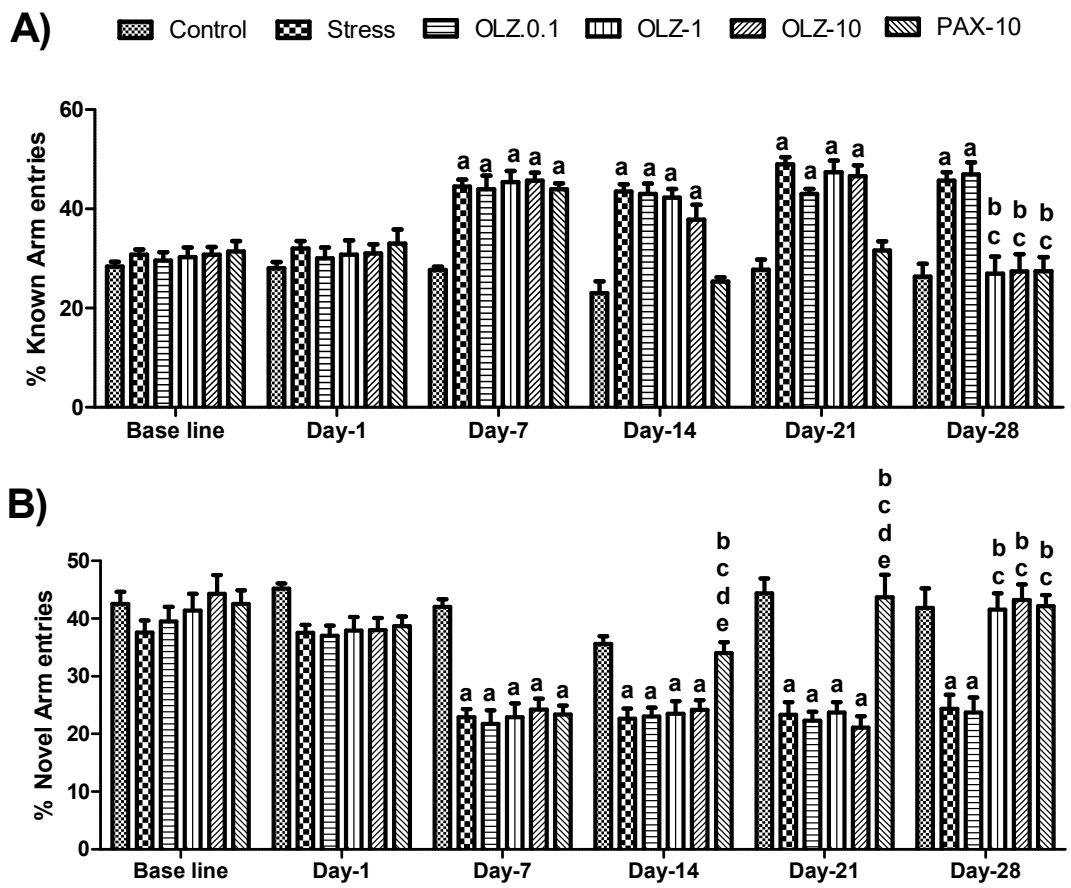

Fig-4.3: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in Y-maze arm discrimination in terms of % known arm entries (A) and % novel arm entries (B) on D-1, D-7, D-14, D-21 and D-28 of the treatment schedule. All the values are Mean±SEM (n=6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS and $^{c}P < 0.05$ compared to OLZ (0.1 mg/kg). [Two-way ANOVA followed by Bonferroni test].

4.3.5 Effect of OLZ on the SRS-induced decline in plasma corticosterone

The effect of OLZ (0.1, 1.0 and 10mg/kg) and PAX (10mg/kg) on SRS-induced alterations in plasma corticosterone is shown in Fig-4.4. One-way ANOVA analysis of the data showed that there were significant differences of plasma corticosterone [F (5, 30) - 66.71; P< 0.05] among the groups on day 28. Post hoc analysis showed that SRS significantly decreased the plasma corticosterone levels compared to control animals. Repeated OLZ (1.0 and 10mg/kg) treatment increased the SRS-induced decline in plasma corticosterone levels. However, OLZ 0.1mg and PAX-10mg showed no effect on SRS-induced changes in plasma corticosterone levels.

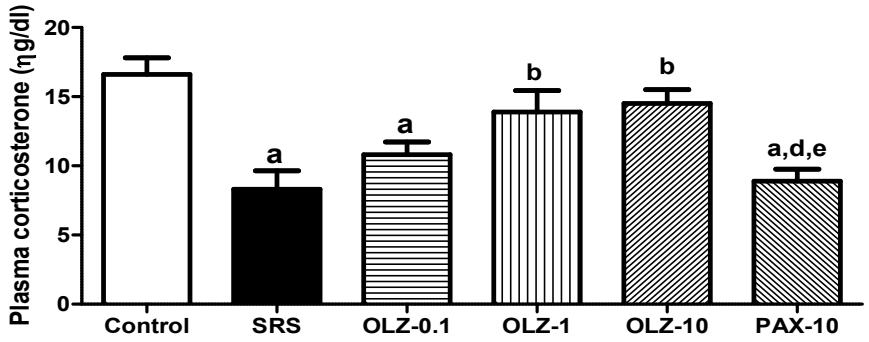

Fig-4.4: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on the levels of plasma corticosterone in SRS subjected rats. All the values are Mean±SEM (n = 6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS control (SRS), $^{c}P < 0.05$ compared to OLZ (0.1 mg/kg), $^{d}P<0.05$ compared to OLZ (1 mg/kg) and $^{e}P < 0.05$ compared to OLZ (10mg/kg). [One-way ANOVA followed by Student Newman-Keuls test].

4.3.6 Effect of OLZ on the expression of BDNF

Fig-4.5 illustrates the effects of repeated OLZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg treatment on the expression of BDNF in different brain regions. Statistical analysis showed that there was significant difference among groups in the level of expression of BDNF in PFC [F (5, 12) - 40.5, P < 0.05] and AMY [F (5, 12) - 40.5, P < 0.05]. Post-hoc analysis

revealed that SRS significantly decreased the expression of the protein BDNF in both PFC and AMY compared to control. Repeated treatment with OLZ in the dose of 1 and 10 mg/kg and PAX-10mg/kg significantly reversed this SRS-induced decline in the expression of BDNF in both the brain regions.

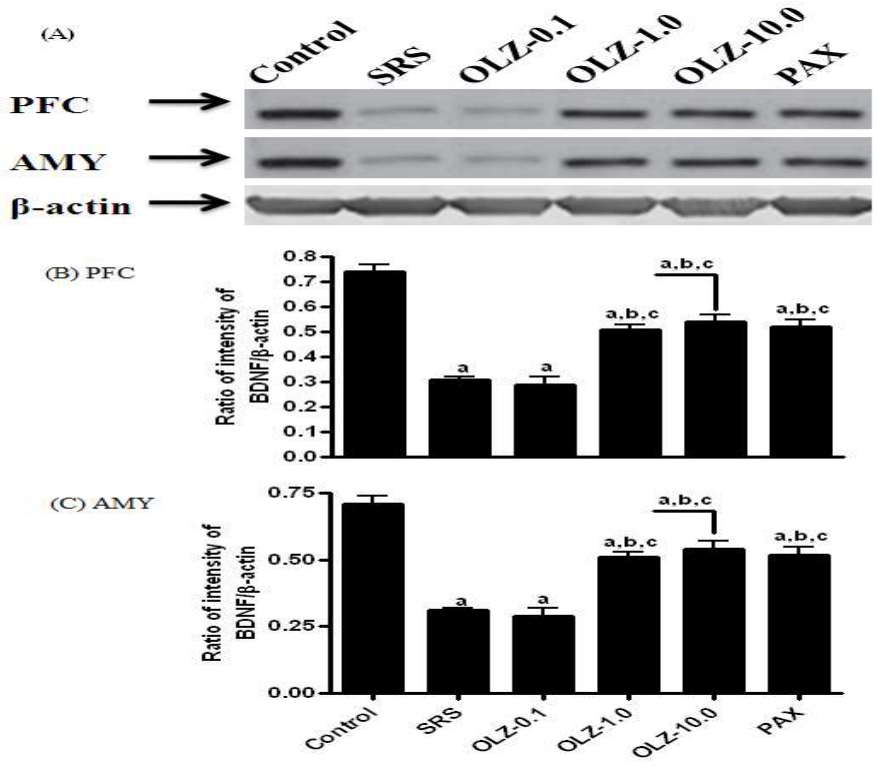

Fig 4.5: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in the expression of BDNF in PFC and AMY (A) Indicates the blot representations of BDNF in PFC and AMY. (B) and (C) is the histogram data expressed as the ratio of the relative intensity of levels of BDNF to β-Actin in PFC and AMY, respectively. All values are expressed as Mean±SEM (n=3). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS and $^{c}P < 0.05$ compared to OLZ (0.1 mg/kg). [One-way ANOVA followed by Student Newman-Keuls test].

4.3.7 Effect of OLZ treatment on the expression of pERK/ERK in PFC

Fig-4.6 illustrates the effects of repeated treatment with OLZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg in the level of expression of pERK and ERK and their ratio in the PFC brain region. Statistical analysis showed that there was a significant difference in the expression of pERK/ERK among groups [F (5, 12) - 26.1 $p<0.05$]. Post-hoc analysis revealed that modified SRS significantly increased the expression of the pERK/ERK in PFC compared to control. Repeated treatment of OLZ in the dose of 1 and 10 mg/kg and PAX-10mg/kg significantly alleviated the SRS-induced enhancement in the expression of pERK/ERK in the PFC OLZ-0.1mg showed no change.

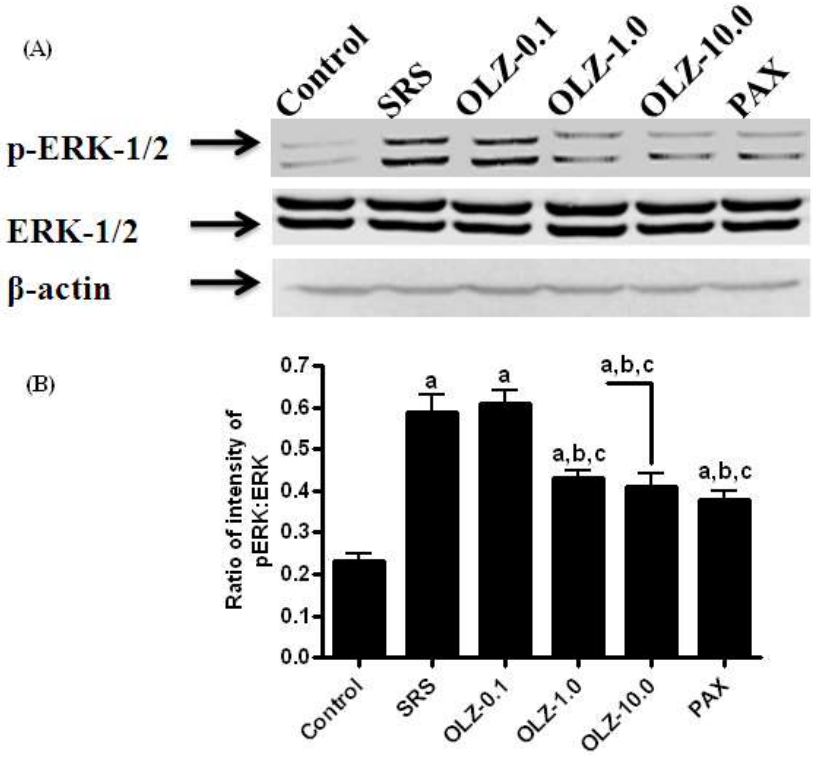

Fig-4.6: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in the expression of ERK in PFC. (A) It consists of the blots representations of levels of pERK and ERK in PFC. The (B) is the histogram data expressed as the ratio of the relative intensity of levels of pERK/ERK in PFC. All values are Mean±SEM (n=3). $^{a}P<0.05$ compared to control, $^{b}P<0.05$ compared to SRS, and $^{c}P< .05$ compared to OLZ (0.1 mg/kg). [One-way ANOVA followed by Student Newman-Keuls test].

4.3.8 Effect of OLZ treatment on the expression of pERK/ERK in AMY

Fig-4.7 illustrates the effects of repeated OLZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg in the level of expression of pERK, ERK and their ratio in the AMY region. As per the statistical analysis, there was a significant difference among groups in the level of expression of pERK/ERK in AMY [F (5, 12) - 25.4, P<0.05]. Post-hoc analysis revealed that SRS significantly enhanced the expression of the pERK/ERK in AMY compared to control. Repeated treatment with OLZ at the doses of 1 and 10 mg/kg and PAX-10mg/kg significantly mitigated this SRS-induced increase in expression of pERK/ERK in the AMY region.

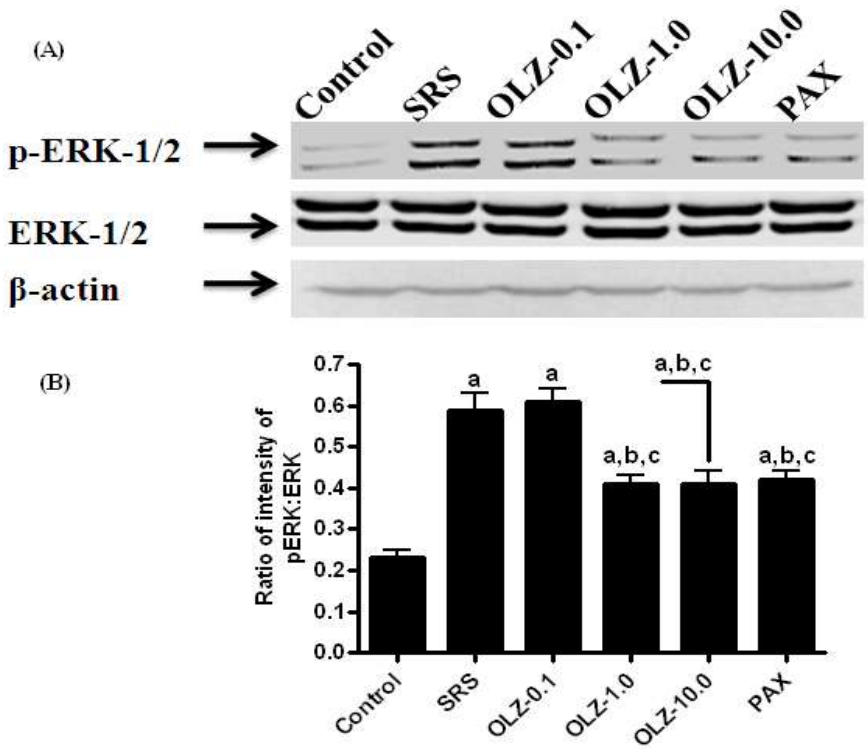

Fig-4.7: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in the expression of ERK in AMY. (A) It consists of the blots, which are representations of the levels of pERK and ERK in AMY. (B) Is the histogram data expressed as the ratio of the relative intensity of levels of pERK/ERK in AMY. All values are Mean±SEM (n=3). ^{a}P <0.05 compared to control, ^{b}P<0.05 compared to SRS and ^{c}P<0.05 compared to OLZ (0.1 mg/kg). [One-way ANOVA followed by Student Newman-Keuls test].

4.3.9 Effect of OLZ on the expression of CREB

The effects of repeated OLZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg in the level of expression of CREB in the two brain regions viz. PFC and AMY are depicted in **Fig-4.8**. Statistical analysis showed significant differences among groups in the degree of expression of CREB in PFC [F (5, 12) - 36.3, P < 0.05] and AMY [F (5, 12) - 35.5, P < 0.05]. Post-hoc the analysis revealed that modified SRS significantly decreased the expression of the CREB in both PFC and AMY compared to control. Repeated treatment of OLZ in the dose of 1 and 10 mg/kg and PAX-10mg/kg significantly reversed the modified SRS-induced decrease in the expression of CREB in both the brain regions.

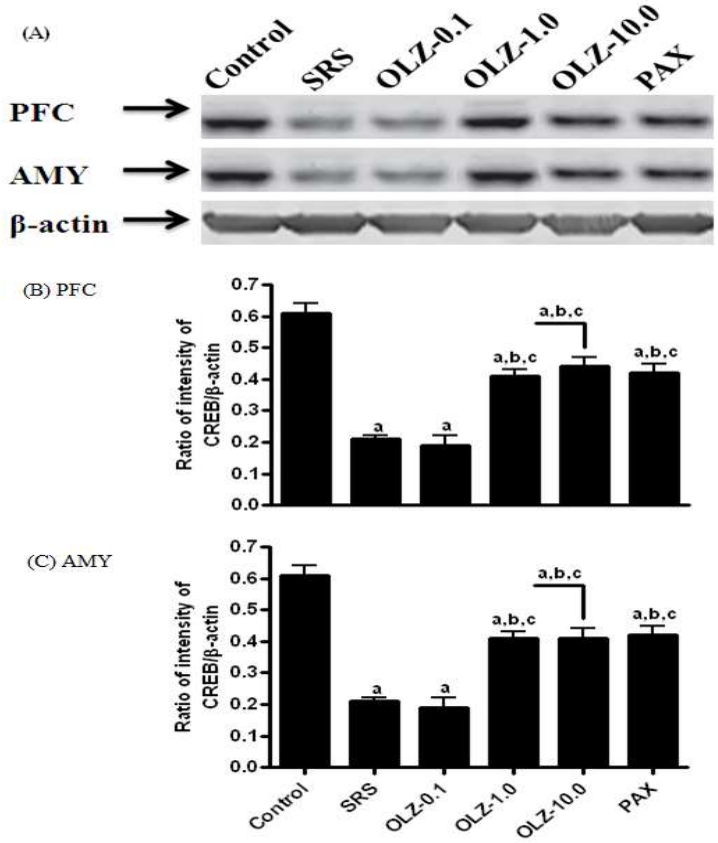

Fig-4.8: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in the expression of CREB in PFC and AMY. (A) Indicates the blots representing the CREB in PFC and AMY. (B), (C) are histograms that express the ratio of the relative intensity of levels of CREB to β-Actin in PFC, AMY, respectively. All values are expressed as Mean±SEM (n=3). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS, and $^{c}P < 0.05$ compared to OLZ (0.1 mg/kg). [One-way ANOVA followed by Student Newman-Keuls test].

4.3.10 Effect of OLZ on the expression of Caspase-3

The effects of repeated OLZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg on the levels of expression of caspase-3 in different brain regions as illustrated in **Fig-4.9**. Statistical analysis of data showed significant differences among groups in the level of expression of caspase-3 in PFC [F (5, 12) - 44.6, P < 0.05] and AMY [F (5, 12) - 30.7, P < 0.05]. Post-hoc analysis revealed that SRS significantly increased the expression of the enzyme caspase-3 in both PFC and AMY compared to control. Repeated treatment of OLZ in the dose of 1 and 10 mg/kg and PAX-10mg/kg significantly reduced the increase in the expression of caspase-3 in both the brain regions compared to the SRS group.

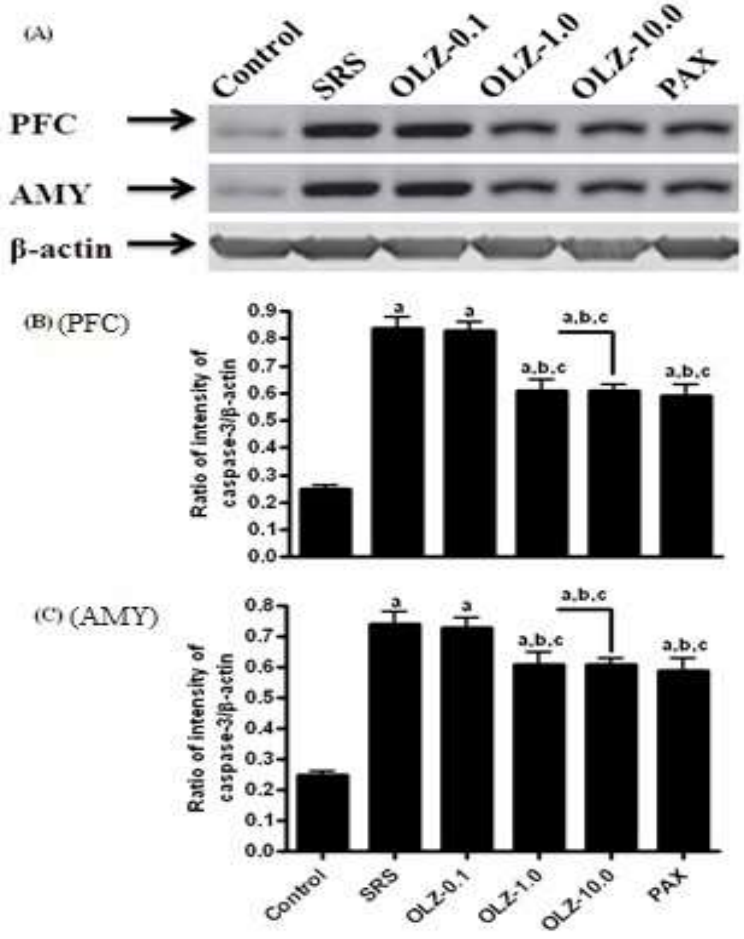

Fig-4.9: The effect of OLZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in the expression of caspase-3 in PFC and AMY. (A) blots are representations of caspase-3 in PFC and AMY. (B) and (C) is the histogram data expressed as the ratio of relative intensity of

levels of caspase-3 to β-Actin in PFC and AMY, respectively. All values are expressed as Mean±SEM (n=3). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS, and $^{c}P < 0.05$ compared to OLZ (0.1 mg/kg). [One-way ANOVA followed by Student Newman-Keuls test].

4.4 Discussion

The salient finding of the study is the anti-PTSD potential of OLZ in the animal model. To the best of our knowledge, this is the first report showing the preclinical anti-PTSD outcome of OLZ. This study also indicates the imperativeness of regulation of the cell signalling factors in the treatment of PTSD. The repeated treatment with OLZ mitigated the modified SRS-induced PTSD-like symptoms. There was an alleviation of the behavioural disturbances brought about by PTSD regarding anxiety and memory. PTSD also disrupts the expression pattern of cell signalling proteins like caspase-3, BDNF, CREB, and ERK. This disruption was also significantly alleviated by repeated OLZ treatment for 28 days.

Animals with PTSD perceive most stimuli as threatening and respond immediately through behavioural and physiological actions, such as freezing. Freezing is a defensive response with symptoms like a reduction in physical movements and heart rate (Fragkaki et al., 2017). This behavioural pattern is thought to be the reason for the sustenance of the disease as it inhibits the adaptive risk assessment of stimuli leading to persistent maladaptive defensive responses. In this study, animals subjected to SRS showed a longer duration of freezing-like response. Repeated drug treatment with OLZ at doses of 1 and 10mg/kg showed a decrease in the duration of freezing on D-21 and D-28. This reduction in freezing due to treatment indicates an enhancement of adaptive risk assessment, which could contribute to the remission of PTSD signs. In another study, OLZ at the doses of 5 and 10mg/kg moderated contextual freezing behaviour in response to shock in ovariectomized rats (Frye et al., 2003). Besides, PAX also mitigated freezing behaviour from D-14 to D-28. This observation was also consistent with other studies wherein chronic administration of PAX showed a decrease in

freezing behaviour in rats subjected to single prolonged stress. However, PAX did not show this effect in acute treatment (Takahashi et al., 2006).

Hyperarousal or anxiety is the primary behavioural symptom observed in patients with PTSD (Ahman et al., 2008). PTSD rats, when exposed to EPM, showed a decline in the number of entries and time spent on the open arm but had enhancement of fecal droppings and immobility period, which are indications of anxiety (Krishnamurthy et al., 2013).

In this study, there was no change in behavioural parameters immediately after variable stress on D-1, but there were significant changes between groups from D-7. Similarly, in a previous study, rats subjected to acute stress showed significant changes in EPM within one hour of acute stress. However, those rats subjected to restress showed changes in the EPM only from D-7 and not immediately after one hour of restress (Harvey et al., 2006). Whereas, in the previous experiment involving the evaluation of risperidone in PTSD, the exposure to SRS showed changes in EPM immediately after variable stress, even before induction of restress (Krishnamurthy et al., 2013). However, these behavioural variations seem to be correlated with the changes in the corticosterone levels in the brain in response to a different stressor (Harvey et al., 2006).

Repeated treatment with OLZ 10mg/kg and PAX-10mg/kg showed to alleviate anxiety-related behaviour regarding the entries and time spent in the open arms, the number of fecal droppings, and also immobility time. Treatment with OLZ-10mg/kg increased the number of entries and time spent on the open arms and also diminished the number of fecal droppings and immobility periods starting from D-21 to D-28. However, OLZ at a dose of 1mg/kg showed only a decrease in fecal droppings from on D-28; but had no effect on open arm entries and time spent. Similar variations in effects were also observed in previous studies wherein at 1mg/kg dose OLZ had mitigating effects on fecal droppings in a stress-induced model of rats (Locchi et al., 2008; Sun et al., 2010). However, at the doses of 10 mg/kg, it

significantly enhanced the entries and time spent in open arms, indicating dose-dependent effects (Frye et al., 2003). These differences in the behavioural effects on EPM could be due to the superior potential of atypical antipsychotics in alleviating fear-related anxiety responses like defecations besides the ability to mitigate intrinsic anxiety (Mead et al., 2008). On the other hand, PAX enhanced the number of entries and time spent in the open arm, besides decreasing the fecal dropping and immobility period from D-14 up to D-28. Anxiolytic properties of paroxetine were also reported earlier for EPM and social fear (Drapier et al., 2007; Toth et al., 2012). However, there were no differences in the total number of entries in between the groups on all the days tested. This is because; the rats in EPM showed enhanced entries into the closed arms despite the decreased entries into open arms leading to a lack of significant change in the total number of entries between groups. Further, there was no development of tolerance to open arm exposures in control group animals due to repeated trials on EPM. This could be due to the time interval gap of 1 week in between exposures. Concurrently in another study, rats exposed to EPM for 18 consecutive days did not show any signs of development of tolerance to the open arms (Treit et al., 1993).

Another distinctive symptom of PTSD is the change in cognitive processes like memory, attention, planning and problem-solving (Hayes et al., 2012). There is predominance to threat detection and interpretation of offensive stimuli as threatening, thereby constricting attention focus at the expense of other cognitive performances (Hayes et al., 2012). In the Y-maze exploration test, SRS caused a gradual loss of exploration behaviour, spatial memory and enhanced anxiety-like behaviour from D-7 up to D-28. These disruptive changes in behaviour were mitigated by OLZ (1.0 and 10mg/kg) and PAX from D-14 and D-21, respectively. In previous studies, OLZ showed to improve spatial learning function and relieve cognitive deficits at doses of 0.1 and 5mg/kg body weights (Hou et al., 2006; Wolff et al., 2003). Even PAX at a dose of 10mg/kg restored the impaired spatial learning and memory in depressed rats (Han et al., 2015). It indicates that both PAX and OLZ (1 and 10mg/kg) improved

memory deficits in PTSD, which is critical for the development of optimal adaptive behaviour.

Another intriguing observation in the study is that there was a decrease in the total number of entries in the Y-maze test. This was because the rats subjected to SRS spent more time in the familiar arm in the direction of the cue with decreased overall movement. Further, the rats in the control group did not show any signs of development of tolerance to the Y maze arms after repeated exposure due to the novelty presented in terms of change in cues for each test.

Research into previous experiments indicates a wide variation in the doses of OLZ tested. At the lower dose range of 0.5 to 2mg/kg body weight, OLZ was found to be effective in anxiety and memory-like mood-related behaviours (Hou et al., 2006; Locchi et al., 2008; Sun et al., 2010). However, others found OLZ to be effective in anxiety and memory in the dose ranges of 5 to 10mg/kg body weight in rats. At 10mg/kg, it showed to have anxiolytic effects in EPM and open field tests and at a dose of 5 mg/kg had effects on memory-related behaviour (Frye et al., 2003; Wolff et al., 2003). However, in contrast to this, few studies also indicated sedative-like effects of OLZ at a dose higher than 2mg/kg (Ahnaou et al., 2003). These dose-dependent variations of effects could result from OLZ's ability to bind to multiple receptors. At regular doses, it binds to 43–80% of dopamine and near saturation of serotonin receptors. Moreover, at doses of 5 to 10mg, OLZ is found to enhance the allopregnanolone (3α-hydroxy-5α-pregnan-20-one) in the brain, which acts as a positive modulator of GABA leading to sedative-like effects (Marx et al., 2000). However, in PTSD, there is a pattern of hyperarousal, which is the cause of anxiety, memory and sleep disturbances. In this study, OLZ at a dose of 10mg/kg had positive implications on anxiety and memory behaviours without any significant sedative effects indicating an insufficient dose. Apart from this, the repeated exposure of rats to forced swim tests (PTSD sensitization) and other behavioural tests performed on the same day could also have hindered OLZ's sedative properties.

In the study, SRS decreased the plasma corticosterone levels; this decrease was in concurrence with other animal studies involving PTSD models (Krishnamurthy et al., 2013). The hypothalmopitutary adrenal axis (HPA) is perturbed in PTSD with increased activity of negative feedback loop of HPA-axis leading to hypocorticosteronemia (De Kloet et al., 2006; Yehuda, 2001). The repeated treatment with OLZ 1 and 10mg/kg mitigated this SRS induced hypocorticosteronemia. However this was not seen with administration of PAX-10mg/kg. This inability of PAX is ascertained to be due to the lack of HPA-axis modulation (Krishnamurthy et al., 2013; Philbert et al., 2012). However, in the clinical studies, the treatment with PAX for 12 weeks to 12 months showed to mitigate this PTSD induced alterations in the plasma cortisol levels. This effect was seen at doses of 20 and 30mg/kg of PAX administration of (Randall et al., 2001). Prolonged administration of PAX leads to desensitization of 5HT1A receptors. This leads to an increased seronergic neurotransmission from raphae nucleus to Paraventricular nucleus of hypothalamus (Randall et al., 2001). The excess serotonin downregulates the CRH mRNA formation leading to decreased synthesis affecting the release of corticosterone from adrenal cortex (Brady et al., 1992). Also, there were significant variations between the baseline cortisol levels and those measured after the stress reminder challenge in PTSD patients. So, the inability of PAX to alleviate the hypocorticosteronemia in SRS could be due to insufficient dose and duration of administration. As far as the we know no preclinical study has reported the plasma corticosterone mitigating effect in PTSD models. However, the atypical antipsychotics do not directly affect the HPA axis but still mitigated the plasma corticosterone levels in SRS model. This effect of antipsychotics is due to their ability to normalize glucocorticoid signaling to bring about normalization of HPA axis (Alice and Marco, 2020).

Repeated OLZ treatment significantly alleviated the PTSD-induced perturbations of cell signalling factors. The pathology of PTSD also involves the disturbance in the cell signalling pathways involving BDNF, CREB, ERK, and caspase-3, as observed in both clinical studies

and experimental models (Andero et al., 2012; Ross, 2009). OLZ at the doses of 1 and 10mg/kg and PAX-10mg/kg enhanced BDNF levels in the PFC and AMY regions. BDNF is a nerve growth factor that is crucial in modulating resilience and vulnerability to stress (Kim et al., 2017). In a stress re-stress model of PTSD in rats, it was shown that repeated administration of OLZ reversed the BDNF and Bcl-2 levels to normal (Luo et al., 2004). This ability of OLZ to enhance BDNF is through its ability to increase basal BDNF gene promoter activity in a dose-dependent manner and also CREB meditated transcription via PKA, PI3K, PKC, and CaMKII signalling pathways (Lee et al., 2010). SRS significantly enhanced the formation of phosphorylated ERK (pERK) over ERK. pERK is predominantly formed under the conditions of stress. A previous study reported that rats subjected to forced swim stress had enhanced pERK levels in the PFC and AMY regions (Shen et al., 2004). The inhibition of ERK phosphorylation leads to a decrease in avoidance behaviour in rats, one of the critical symptoms of PTSD (Whitaker et al., 2016). Further, increased signalling of ERK regulates gene expression, CREB formation, neuronal plasticity and memory (Davis et al., 2006; Shen et al., 2004; Sweatt, 2001). The increased formation of pERK would activate the pro-apoptotic transcription factor CHOP gene (C/EBP-homologous protein) (Harding et al., 2001). It implies that the enhancement of ERK formation would help in the correction of neuronal plasticity and memory deficits. On the other hand, the minimization of pERK formation prevents the activation of apoptotic factors. In this study, repeated treatment by OLZ (1and 10mg/kg) and PAX-10mg/kg alleviated the formation of pERK.

CREB is a transcription factor involved in the neuronal process of learning, neuronal plasticity, and modulation of stress responses. Patients with PTSD were found to have lower levels of total CREB protein in their blood samples (Martini et al., 2013). In this experiment, repeated administration of OLZ (1 and 10mg/kg) and PAX-10mg/kg showed a significant up-regulation of CREB expression, which was decreased due to SRS in both the PFC and AMY. This result is similar to another study involving the assessment of mood-stabilizing effects of

OLZ. Four weeks of administration of lithium and OLZ showed a significant up-regulation of CREB in rats (Hammonds et al., 2009). Also, PAX at a dose of 10mg/kg was found to up-regulate CREB expression in a rat model of depression (Han et al., 2015). It indicates that the repeated drug administration could have modulated PTSD-induced derangements through enhancement of CREB.

Apoptotic factors like Caspase-3, 9, and cytochrome C oxidase act as the contributing factors for the development of PTSD (Garabadu et al., 2015; Han et al., 2013). Caspase-3 is one of the critical enzymes involved in the downstream pathway of apoptotic cell death. SRS significantly increased caspase-3 in both PFC and AMY regions of the brain. In a model of PTSD involving single prolonged stress, there was increased expression of caspase-3 and 9 (Zhang et al., 2016). Repeated treatment with OLZ (1 and 10mg/kg) and PAX (10mg/kg) significantly diminished the increase in expression of caspase-3 in both regions. Previous studies also support the modulating effect of OLZ on apoptosis (Wang et al., 2005). Another antipsychotic, risperidone, also showed neuroprotective effects by inhibiting the activation of caspases (Garabadu et al., 2015; Ukai et al., 2004). Similarly, PAX at a dose of 10mg/kg reduced the hippocampal expression of caspase-3 in a rat model of chronic mild stress (Khedr et al., 2015).

From the above discussion, it can be inferred that BDNF enhancement leads to the extinction of fear and memory consolidation in PTSD through the stimulation of the ERK signalling cascade (Andero et al., 2012; Ji et al., 2016). This ERK, in turn, translocates to the nucleus and phosphorylates the CREB to mediate downstream transcriptional activation, which further modulates the memory consolidation (Garabadu et al., 2015; Kida et al., 2002; Sgambato et al., 1998). On the other side, the decreased expression of pERK prevents the activation of apoptotic factors like the caspase-3 resulting in the promotion of cell survival pathways (McKay et al., 2007). Thus the OLZ treatment could have the potential to minimize

cell death, enhance plasticity, mitigate anxiety and memory disturbances in PTSD rats.

However, the classical theory holds that atypical antipsychotics like OLZ produce therapeutic effects through their action on the serotonergic system. Hence, its therapeutic potential through the cell signalling systems can be contrasting. Still, studies have found that there exists a synergistic mechanism between serotonin and BDNF systems in affective behaviours (Martinowich et al., 2008). Serotonergic cells have been found to enhance BDNF function and pretreatment with a 5-HT2A receptor antagonist can prevent the stress-induced decrease in BDNF ((Madhav et al., 2001; Vaidya et al., 1997). Also, SSRIs which enhance synaptic serotonin levels, reverse the stress-induced down-regulation of BDNF gene expression (Autry et al., 2012; Gonul et al., 2005; Madhav et al., 2001; Vaidya et al., 1997). Hence, these drugs acting through the serotonergic pathway could yet be involved in the activation of the BDNF related pathway (Martinowich et al., 2008).

Also, the requirement of prolonged drug treatment in the clinic and their ability to enhance neurotrophic factors indicate that these drugs have a notable effect on cell survival than the monoamine pathway alone.

Thus from the above study, it appears that OLZ has potential anti-PTSD properties comparable to PAX in terms of both behavioural improvements and also neurotrophic enhancement. However, the effect of PAX seems to start earlier, as seen by the improvement in behavioural parameters on D-14, while that of OLZ's starts from D-21. Therefore, the study shows the preclinical potential of OLZ in the treatment of PTSD.