5.1 Introduction

Aripiprazole is a first-line atypical antipsychotic used for the treatment of episodes of schizophrenia. Interestingly, it was also found to be effective in the mitigation of symptoms of PTSD (Britnell *et al.*, 2017). However, APZ has not been explored in the animal models of PTSD for its effectiveness. A study on animal models would help to understand the mechanism and further ascertain the claims. Atypical is found to act on multiple receptors of dopamine, serotonin, and norepinephrine as per their receptor binding profile. Besides, they are also found to be acting on cell signalling mechanisms. Further, APZ having antidepressant properties could be more beneficial in the treatment of PTSD with symptoms of depression (Kim *et al.*, 2015).

Short-term effects of antipsychotic drugs are closely related to their receptor binding properties, whereas the repeated administration of these drugs could lead to improvement of functions like cognition through a series of adaptive mechanisms (Luoni *et al.*, 2014a). In a rat model of acute stress, chronic administration of APZ showed to enhance BDNF levels in the PFC and hippocampus (Luoni *et al.*, 2014a). The signalling system associated with BDNF is the critical target for APZ (Park *et al.*, 2009). Chronic APZ administration showed to enhance CREB in normal rat brains (Pan *et al.*, 2016). APZ is found to inhibit cell death in cultured dopaminergic neurons and also apoptosis in ischemic mice brains (Gil *et al.*, 2018; Shioda *et al.*, 2015). This ability of APZ is found to be due to its agonist action on D2 receptors leading to enhanced BDNF expression as well as activation of calcium-calmodulin-dependent protein kinase-II (Shioda *et al.*, 2015). APZ is also found to modulate ERK, which can play a vital role in the development and maintenance of PTSD in rats (Whitaker *et al.*, 2016). In previous studies, OLZ showed neuroprotective effects through the enhancement in BDNF and CREB. This is due to its ability to up-regulate the gene transcription, which

further enhances CREB formation (Lee *et al.*, 2010; Park *et al.*, 2013). Apart from the promotion of cell survival factors, OLZ also blocked the activation of caspase-3, an apoptotic cell death factor (Wang *et al.*, 2005).

5.2 Materials and methods

5.2.1 Drugs & chemicals

APZ was generous as a gift sample from Micro Laboratories, India Ltd. Paroxetine (PAX) was procured as a gift sample from Ranbaxy Laboratories, India. Both of them were insoluble in water and suspended in 0.5% carboxymethylcellulose (CMC) solution. The antibodies required for the western blot procedure were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, California, USA). All other chemicals and reagents were obtained from local suppliers.

5.2.2 Animals

The adult male rats of Charles Foster strain weighing between 220-260g were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University. These were housed in polypropylene cages with 6 in each at a suitable temperature of 25 ± 1^{0} C and 45–55% RH. They had free access to food and water throughout the experiment. The experimental procedures were conducted between 08:00 and 14:00h. All possible care was taken to minimize the suffering and the total number of animals used. The principles of laboratory animal care as per (National Research Council US Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011) guidelines were strictly followed. The experiments on the animals were performed with approval from the animal ethical committee (Ref No. Dean/10-11/148).

5.2.3 Experimental protocol

The animals were assigned into five groups with 6 in each as group-1 (control), group-2 (PTSD control), group-3, 4, and 5 (APZ doses as 0.1mg/kg, 1.0 mg/kg and 10 mg/kg respectively) and group-6 (PAX-10mg/kg) (Biojone *et al.*, 2010). The experiment was conducted for 28 days and drug treatment was given for 27 days, i.e., starting from the day after initial stress exposure, as shown in Fig 5.1. The control and PTSD control rats received plain 0.5% CMC suspension. While the rats of treatment groups 3, 4 and 5 received orally 0.1, 1.0 and 10mg/kg doses of APZ suspensions. Their behavioural patterns in terms of freezing, anxiety, and memory were conducted on day-0, 2, 7, 14, 21 and 28th day of the treatment schedule. Animals were decapitated on the last day, i.e., day-28; the blood was collected and the brain regions were separated and stored at -80°C until further biochemical assessments were carried out.

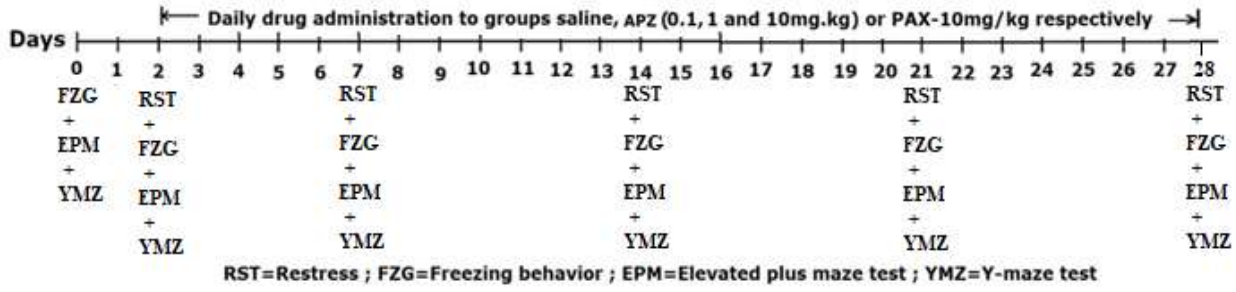

Fig 5.1. Schematic diagram of the experimental protocol

5.2.4 Stress-restress (SRS)

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). Briefly, on Day-2, animals were subjected to a single session of prolonged stress for 2hrs in a metallic restrainer. Then immediately subjected to forced swim test in an 18cm swim tank at an ambient temperature of 25^{0}C for about 20mins. Animals were allowed to recover for 15min and then again exposed to 0.8ml of 4% halothane vapours (stress) until loss of consciousness. Then animals were returned to their home cages. Again on every seventh day,

animals were again exposed to 20 min swim stress (restress) to enhance the sensitization.

5.2.5 Evaluation of freezing

Contextual freezing measurement was performed on all animals by exposing them to the reminder situation for 5min on days 0, 2, 7, 14, 21 and 28, respectively. Freezing is defined as a behaviour with the absence of all movements scored during the situational reminder. The total cumulative time spent by the animal in freezing was determined and scored by video tracking software.

5.2.6 Evaluation of anxiety

Animals were evaluated for their anxiety symptoms using elevated plus-maze on days 0, 2, 7, 14, 21 and 28 after the re-stress procedure in PTSD-induced rats. The number of entries and also the time spent on the open and enclosed arms were recorded during the next 5 minutes using a video tracking system. When all four paws of the rat were inside the arm, an arm entry was counted. The behavioural parameters were further observed by a person unaware of the entire experimental protocol.

5.2.7 Evaluation of Memory

The defects in memory of PTSD rats were evaluated using the Y-maze test on the day as mentioned in a schematic representation. Y-maze is primarily used to assess spatial recognition memory. The arms were designated as starting arm, known arm, and a novel arm. In the first trial, the novel arm entry was blocked and the rats were allowed to move within the other two arms for 15 minutes. They were again exposed to Y-maze after about four hours and now the animals were allowed to access all three arms for 5 min. The number of entries into each arm was recorded for a 5-min period. The behavioural parameters like the total number of entries into all the arms (for the 5 min of trials 1 and 2), the % 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 trials 1 and 2) is indicative of general exploration attitude

(curiosity) and the % entries in known versus novel arm for the 5 min period of trial two was considered as a measure of arm discrimination (spatial recognition memory).

5.2.8 Estimation of plasma corticosterone by HPLC

The levels of plasma corticosterone (CORT) were measured by using High-performance liquid chromatography (HPLC) connected to a UV detector at a wavelength of 250nm (Waters, USA) (Krishnamurthy *et al.*, 2013). Dexamethasone was used as an internal standard and the mobile phase consisted of methanol: water (70:30) at a flow rate of 1.2 ml/min. CORT was detected at 250nm and the chromatogram was recorded and analyzed with Empower software.

5.2.9 Western blot analysis

Briefly, the brain tissues were lysed in a buffer containing a complete protease inhibitor cocktail. 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 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 and 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 corresponding secondary antibodies. An immunoreactive band of proteins was detected by chemiluminescence using enhanced chemiluminescence (ECL) reagents (Amersham

Bioscience, USA). The densitometric scan of films quantified the results. The immunoreactive area was determined by densitometric analysis using Biovis gel documentation software.

5.2.10 Statistical analysis

The data were analyzed statistically using GraphPad Prism version-5. Plasma corticosterone and the data from western blot studies were analyzed using one-way ANOVA. All the remaining parameters were analyzed by repeated measure two-way ANOVA.

5.3 Results

5.3.1 Effect of APZ on open arm entries in EPM

The effect of repeated administration of APZ (0.1, 1.0 and 10 mg/kg) on the open arm entries in Y-maze was illustrated in Fig-5.2. Analysis by Two-way ANOVA showed that there were significant differences among groups in open arm entries [F (5, 180) = 65.83, $p<0.05$], time [F (5, 180) = 69.64, $p<0.05$], and their interaction [F (25, 180) = 5.80, $p<0.05$], The post-hoc test showed that the SRS caused changes in open arm entries from D-7 to D-28 compared to control rats, and this effect was also reversed by treatment with APZ. APZ treatment increases the open arm entries in Y-maze.

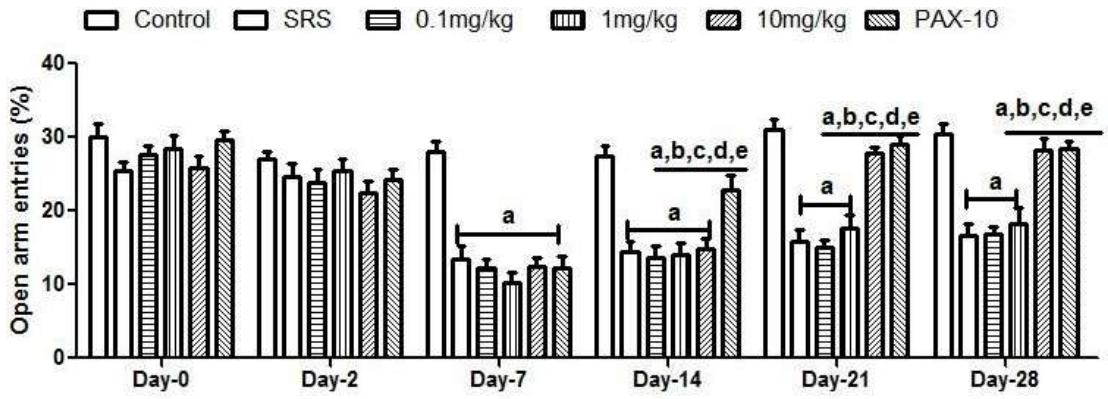

Fig 5.2: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX (10.0 mg/kg) on time spent in open arm in EPM on days-2, 7, 14, 21, 28 respectively. All values are Mean SEM (n = 6).). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to modified SRS control (SRS), $^{c}P < 0.05$ compared to APZ (0.1 mg/kg), $^{d}P < 0.05$ compared to APZ (1 mg/kg) and $^{e}P < 0.05$ compared to APZ (10 mg/kg) [Repeated measure two-way ANOVA followed by Bonferroni test].

5.3.2 Effect of APZ on open arm time spent on EPM

The effect of repeated administration of APZ (0.1, 1.0 and 10 mg/kg) on the open arm time spent in Y-maze was illustrated in Fig-5.3. Analysis by Two-way ANOVA showed that there were significant differences among groups in open arm time spent [F (5, 180) = 38.45, $p<0.05$], time [F (5, 180) = 24.60, $p<0.05$], and their interaction [F (25, 180) = 4.24, $p<0.05$], The post-hoc test showed that the SRS caused changes in open arm time spent from D-7 to D-28 compared to control rats, and this effect was also reversed by treatment with APZ. APZ treatment increases the open arm time spent in Y-maze.

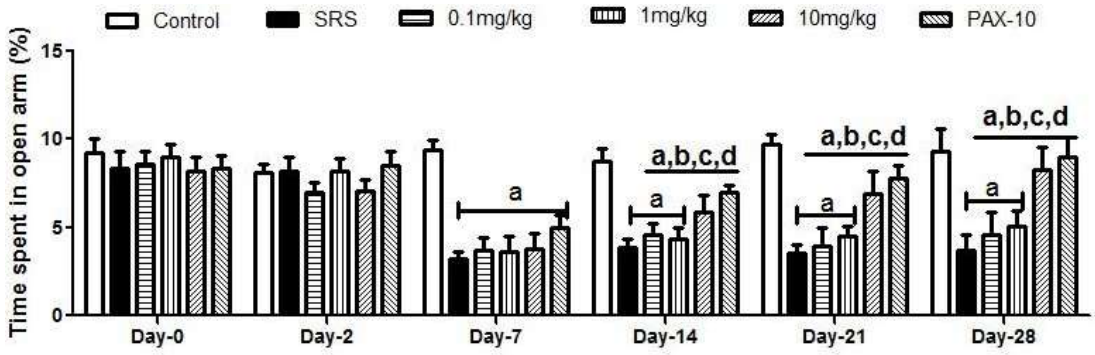

Fig 5.3: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX (10.0 mg/kg) on open arm entries in EPM on days-2, 8,14,20,26 respectively. All values are Mean SEM (n = 6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to modified SRS control (SRS), $^{c}P < 0.05$ compared to APZ (0.1 mg/kg) and $^{d}P < 0.05$ compared to APZ (1 mg/kg) [Repeated measure two-way ANOVA followed by Bonferroni test].

5.3.3 Effect of APZ on fecal pellets on EPM

The effect of repeated administration of APZ (0.1, 1.0 and 10 mg/kg) on the fecal pellets on EPM was illustrated in Fig-5.4. Analysis by Two-way ANOVA showed that there were significant differences among groups in fecal pellets [F (5, 180) = 63.78, $p<0.05$], time [F (5, 180) = 57.62, $p<0.05$], and their interaction [F (25, 180) = 10.48, $p<0.05$], The post-hoc test showed that the SRS caused changes in fecal pellets from D-7 to D-28 compared to control rats, and this effect was also reversed by treatment with APZ. APZ treatment decreases the fecal pellets in EPM.

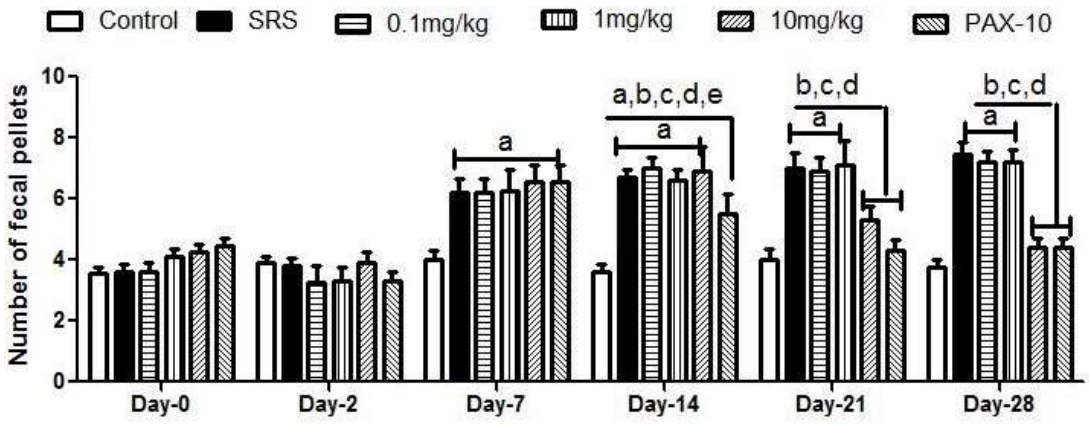

Fig 5.4 The effect of APZ (0.1, 1 and 10 mg/kg) and PAX (10.0 mg/kg) on fecal dropping in EPM on days-2, 8,14,20,26 respectively. All values are Mean SEM (n = 6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to modified SRS control (SRS), $^{c}P < 0.05$ compared to APZ (0.1 mg/kg) and $^{d}P < 0.05$ compared to APZ (1 mg/kg). [Repeated measure two-way ANOVA followed by Bonferroni test].

5.3.4 Effect of APZ on immobility period

The effect of repeated administration of APZ (0.1, 1.0 and 10 mg/kg) on the immobility period on EPM was illustrated in Fig-5.5. Analysis by Two-way ANOVA showed that there were significant differences among groups in the immobility period [F (5, 180) = 101.12, $p<0.05$], time [F (5, 180) = 139.8, $p<0.05$], and their interaction [F (25, 180) = 17.76, $p<0.05$], The post-hoc test showed that the SRS caused changes in the immobility period from D-7 to D-28 compared to control rats, and this effect was also reversed by treatment with APZ. APZ treatment decreases the immobility period in EPM

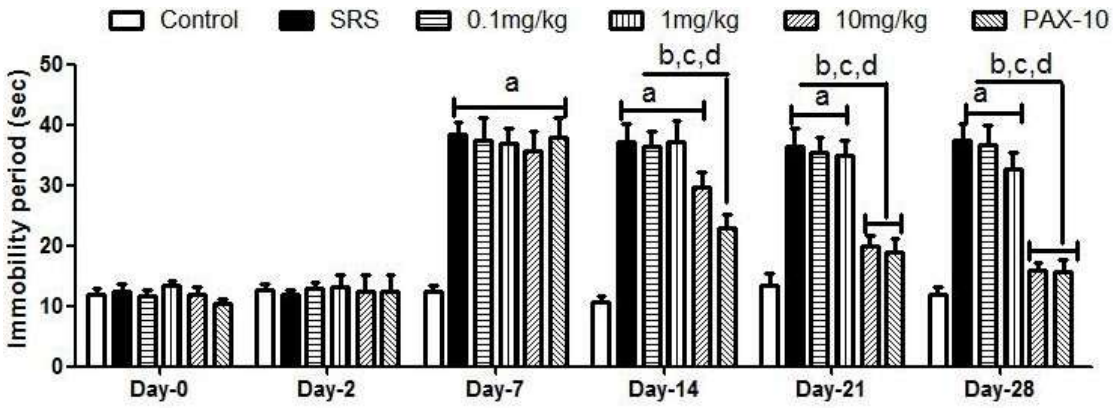

Fig-5.5. The effect of APZ (0.1, 1 and 10 mg/kg) and PAX (10.0 mg/kg) on time spent in open arm in EPM on days-2, 8,14,20,26 respectively. All values are Mean±SEM (n = 6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to modified SRS control (SRS), $^{c}P < 0.05$ compared to APZ (0.1 mg/kg) and $^{d}P < 0.05$ compared to APZ (1 mg/kg) [Repeated measure two-way ANOVA followed by Bonferroni test].

5.3.5 Effect of APZ on Y-maze Trail-I

The effect of repeated APZ (0. 1, 1.0 and 10mg/kg) and PAX-10mg/kg treatment on SRS-induced changes in exploratory behaviour (curiosity) in trial-1 was presented in figure-5.6. Analysis with repeated measure two-way ANOVA showed significant differences of curiosity in trial-1 among groups ([F (5, 180) - 80.15; $P < 0.05$], time ([F (5,180) - 151.9; $P < 0.05$] and an interaction between group and time ([F (25, 180) - 19.82; $P < 0.05$]. 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. Treatment with APZ (10mg/kg) showed a significant increase in the total arm entries on day 28, whereas PAX treatment showed a significant increase in total arm entries on day 14, 21, and 28.

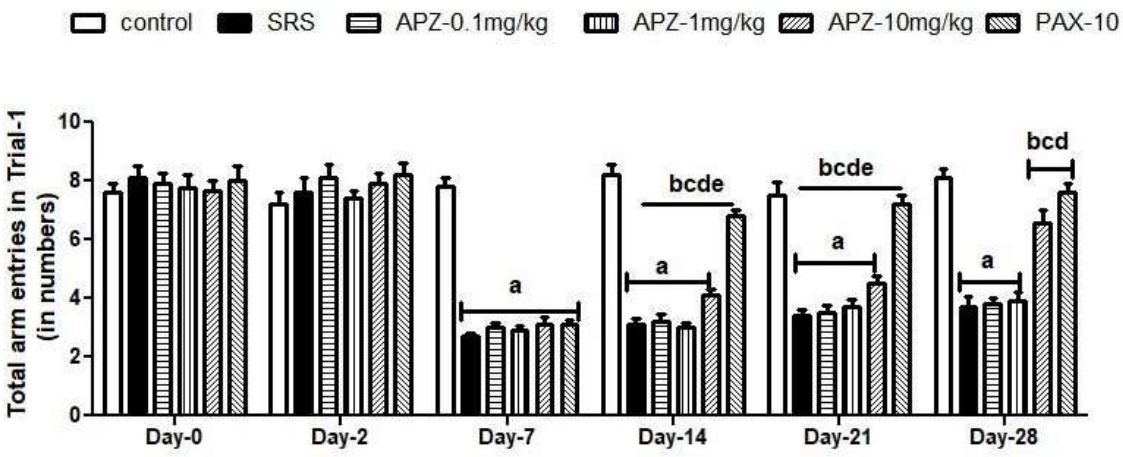

Fig 5.6. The effect of APZ (0.1, 1 and 10 mg/kg) and PAX (10.0 mg/kg) on total arm entries trial-I in Y-maze on days-2, 8, 14, 20, 26 respectively. All values are Mean±SEM (n = 6). [a]$P < 0.05$ compared to control, [b]$P < 0.05$ compared to modified SRS control (SRS), [c]$P < 0.05$ compared to APZ (0.1 mg/kg), [d]$P < 0.05$ compared to APZ (1 mg/kg) and [e]$P < 0.05$ compared to APZ (10 mg/kg) [Repeated measure two-way ANOVA followed by Bonferroni test].

5.3.6 Effect of APZ on Y-maze Trail-II

The effect of repeated APZ (0. 1, 1.0 and 10mg/kg) and PAX-10mg/kg treatment on SRS-induced changes in exploratory behaviour (curiosity) in trial-2 was presented in fig. 5.7. Analysis with repeated measure two-way ANOVA showed significant differences of curiosity in trial-2 among groups [F (5, 180) - 17.8;P < 0.05] respectively), time [F (5, 180) - 36.54; P < 0.05] and an interaction between group and time [F (25, 180) - 2.942; P < 0.05].Treatment with APZ (10mg/kg) showed significant increase in the total arm entries on day 28, where as PAX treatment shown significant increase in total arm entries on day 14,21, and 28.

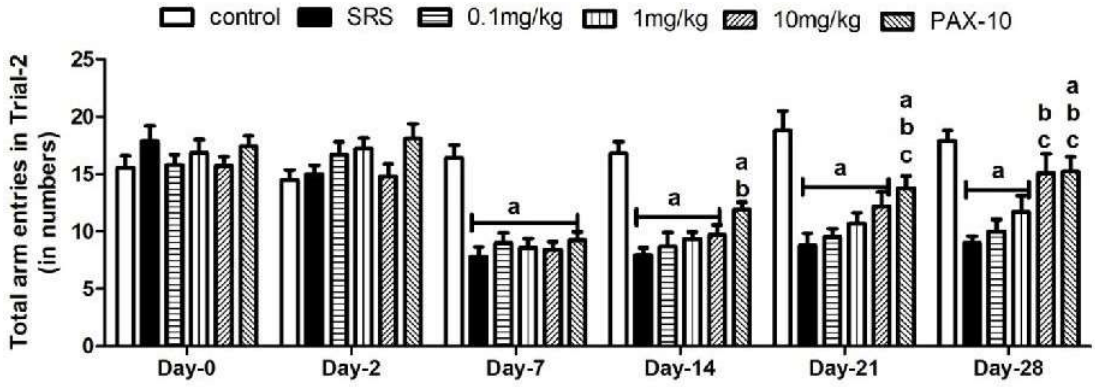

Fig 5.7: The effect of APZ (0.01, 0.1 and 1.0 mg/kg) and PAX (10.0 mg/kg) on total arm entries trial-II in Y-maze on days-2, 8, 14, 20, 26 respectively. All values are Mean SEM (n= 6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to modified SRS control (SRS) and $^{c}P < 0.05$ compared to APZ (0.1 mg/kg) [Repeated measure two-way ANOVA followed by Bonferroni test].

5.3.7 Effect of APZ on SRS-induced plasma corticosterone level

Fig. 5.8 represents the plasma corticosterone levels in PTSD rats. Statistical analysis of the data shows significant differences among groups [F (5, 35) - 66.47, P < 0.05]. Post hoc analysis by using Neuman Keuls indicates the SRS decreases the plasma corticosterone levels. APZ increased the plasma corticosterone levels decreased by SRS at a dose of 10mg/kg. However, PAX did not show the effect on plasma corticosterone.

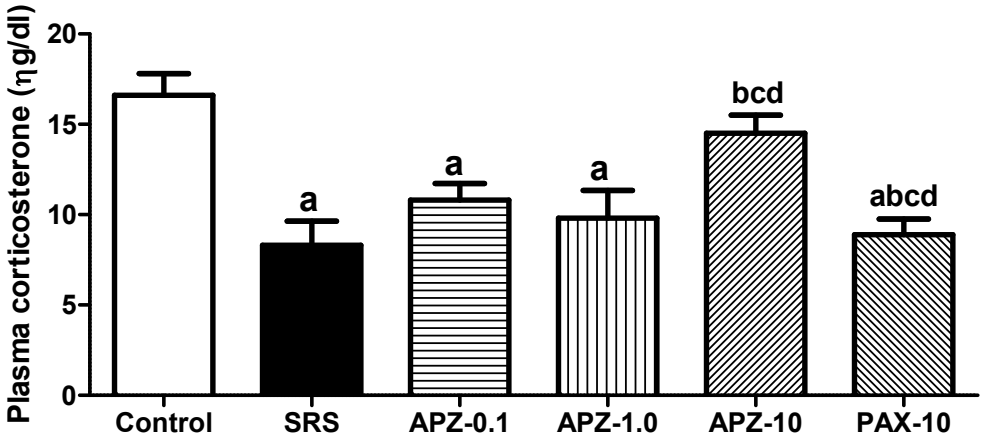

Fig 5.8: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS-induced changes in plasma corticosterone level. All the values are Mean ± SEM (n=6). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS, $^{c}P < 0.05$ compared to APZ (0.1 mg/kg), and $^{d}P < 0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

5.3.8 Effect of APZ on SRS-induced brain serotonin levels

The table-5.1 represents the effect of SRS on 5-HT levels in PFC and AMY. Statistical analysis of the data shows significant differences among groups PFC [F (5, 30) – 1.24, P < 0.05] AMY [F (5, 30) – 116.2, P < 0.05]. Post hoc analysis by using Neuman Keuls indicates the SRS increased the 5HT levels compared with control. APZ treatment decreased the 5HT levels in PFC and AMY. However, PAX did not show any significant effect on 5HT levels.

Table 5.1 APZ Effect on 5HT in SRS rats brain regions PFC and AMY

Region	Control	SRS	APZ 0.1mg/kg	APZ 1mg/kg	APZ 10 mg/kg	PAX 10 mg/kg
PFC	5.15± 0.42	6.04 ± 0.41	5.31 ± 0.54	5.62 ± 0.69	6.08 ± 1.03	5.1 ± 0.79
AMY	15.68 ± 0.53	25.2 ± 0.41[a]	25.42 ± 2.12	21.13 ±1.2	17.24 ± 2.14[a,d]	25.5 ± 1.16[a,d]

The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the levels of 5-HT in PFC and AMY. [a]P < 0.05 compared to control, [d]P < 0.05 compared to APZ 1mg/kg. All values are expressed as Mean ± SEM (n=3) [One-way ANOVA followed by Student Newman-keuls test].

5.3.9 Effect of APZ on SRS-induced brain dopamine levels

Table 5.2 represents the effect of SRS on DA levels in PFC and AMY. Statistical analysis of the data shows no significant differences among groups PFC [F (5, 30) – 0.24, P < 0.05] AMY [F (5, 30) – 0.53, P < 0.05]. Post hoc analysis by using Neuman Keuls indicates the SRS increased the 5HT levels compared with control. APZ treatment did not change the DA levels in PFC and AMY. However, PAX did not show any significant effect on DA levels.

Table 5.2 APZ effect on DA in SRS induced rat brain regions PFC and AMY

Region	Control	CRS	APZ 0.1mg/kg	APZ 1mg/kg	APZ 10 mg/kg	PAX 10 mg/kg
PFC	0.75 ± 0.16	1.05 ± 0.15	1.2 ± 0.2	1.0 ± 0.16	0.87 ± 0.5	0.65 ± 0.16
AMY	2.14 ± 0.1	2.49 ± 0.16	2.2 ± 0.34	2.33 ± 0.13	2.14 ± 0.36	2.62 ± 0.20

The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the levels of 5-HT in PFC and AMY. All values are expressed as Mean ± SEM (n=3) [One-way ANOVA followed by Student Newman-keuls test].

5.3.10 APZ induces the expression of BDNF

Fig-5.9 illustrates the effects of repeated APZ (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) - 42.6, P < 0.05] and AMY [F (5, 12) - 47.4, 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 APZ in the dose of 10 mg/kg and PAX-10mg/kg significantly reversed this SRS-induced decline in the expression of BDNF in both the brain regions.

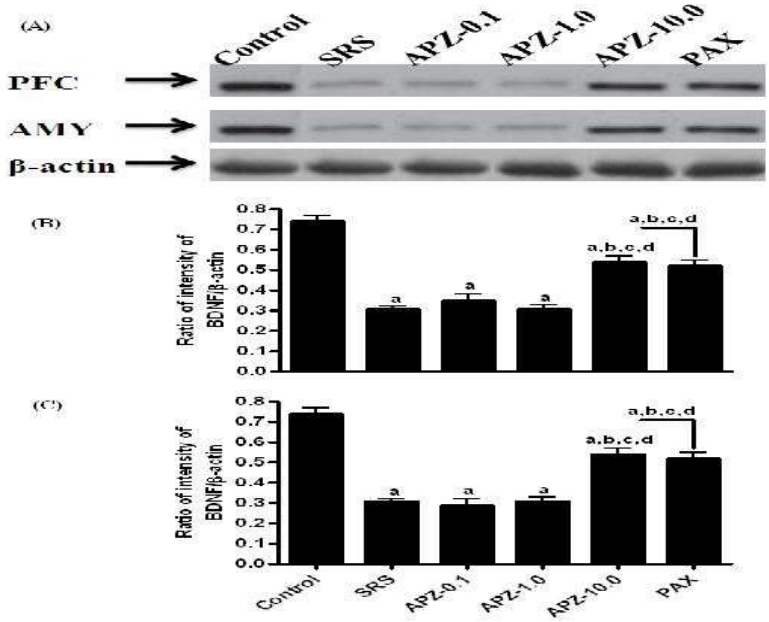

Fig 5.9: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the expression of BDNF in PFC and AMY. (A) Indicates the blot representations of BDNF in PFC and AMY. (B) and (C) is the histogram data expressed as the ratio of the relative intensity of levels of BDNF to β-Actin in PFC 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, $^{c}P < 0.05$ compared to APZ (0.1 mg/kg), and $^{d}P < 0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

5.3.11 Effect of APZ treatment on the expression of pERK in PFC

Fig-5.10 indicates the effects of repeated treatment with APZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg in the level of expression of ERK and p-ERK in the PFC. Statistical analysis showed that there was a significant difference of treatment among [F (5, 12) - 37.1, P < 0.05]. Post-hoc analysis revealed that modified SRS significantly increased the expression of the p-ERK in PFC compared to control. Repeated treatment with APZ at the dose of 10 mg/kg and PAX-10mg/kg also significantly mitigated the SRS-induced expression of p-ERK in the PFC.

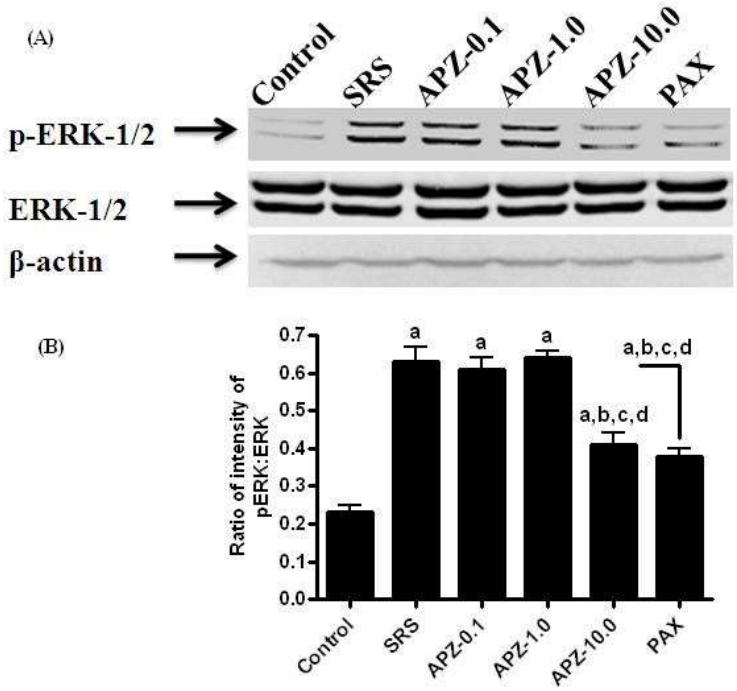

Fig 5.10. The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the expression of ERK in PFC. (A) It consists of the blots representations of levels of p-ERK/ERK ratio in PFC. The (B) is the histogram data expressed as the ratio of the relative intensity of levels of pERK/ERK in PFC. All values are Mean ± SEM (n=3). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS, $^{c}P < 0.05$ compared to APZ (0.1 mg/kg) and $^{d}P < 0.05$ compared to APZ (1mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

5.3.12 Effect of APZ treatment on the expression of pERK in AMY

The effects of repeated APZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg in the level of expression of ERK and pERK in the AMY region is illustrated in Fig-5.11. The statistical analysis showed a significant difference among groups in the level of expression of p-ERK in AMY [F (5, 12) - 28.9, $P < 0.05$]. The post-hoc analysis revealed that SRS significantly enhanced the expression of the p-ERK in the AMY compared to the control. However, the repeated treatment with APZ at the doses of 10 mg/kg and PAX-10mg/kg significantly mitigated this SRS-induced increase in expression of p-ERK levels in the AMY region.

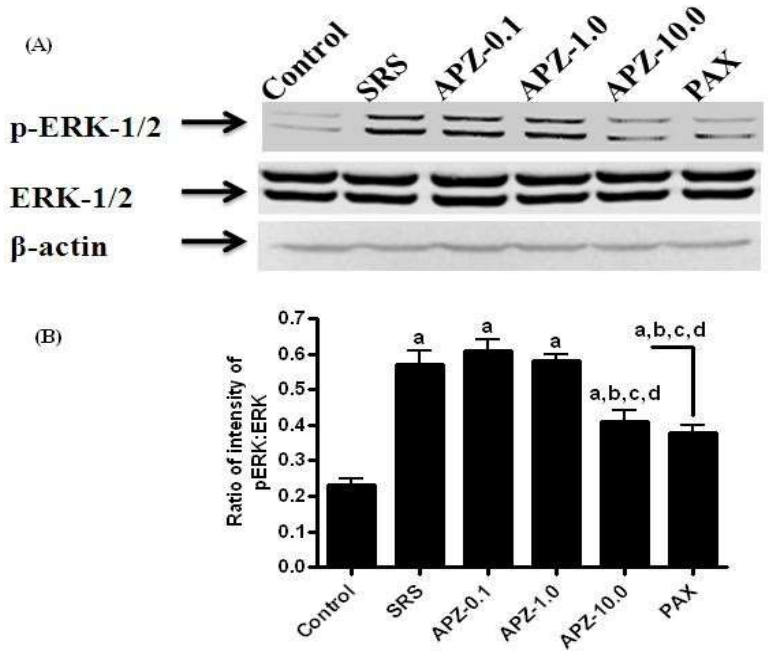

Fig 5.11: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the expression of ERK in AMY. (A) It consists of the blots representations of levels of p-ERK/ERK in AMY. The (B) is the histogram data expressed as the ratio of the relative intensity of levels of pERK/ERK in AMY. All values are Mean ± SEM (n=3). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to SRS, $^{c}P < 0.05$ compared to APZ (0.1 mg/kg), and $^{d}P < 0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

5.3.13 APZ promotes the expression of CREB

The effects of repeated APZ (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-5.12. Statistical analysis showed significant differences among groups in the degree of expression of CREB in PFC [F (5, 12) - 44.4, P < 0.05] and AMY [F (5, 12) - 42.6, P < 0.05]. Post-hoc the analysis showed that modified SRS significantly decreased the expression of the CREB in both PFC and AMY compared to control. Repeated treatment of APZ in the dose of 10 mg/kg and PAX-10mg/kg significantly reversed the modified SRS-induced decrease in the expression of CREB in both the brain regions.

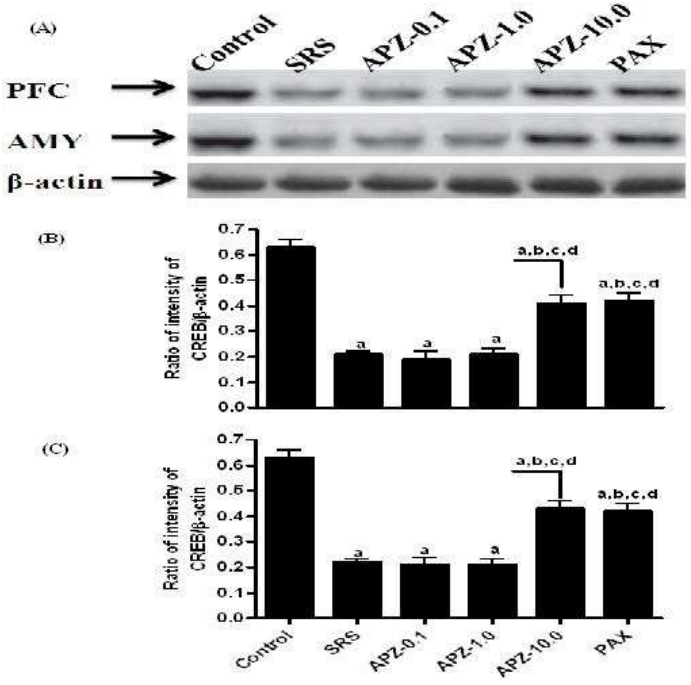

Fig 5.12: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the expression of CREB in PFC and AMY. (A) Indicates the blot representations of CREB in PFC and AMY. (B) and (C) is the histogram data expressed as the ratio of the relative intensity of levels of CREB to β-Actin in 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, $^{c}P < 0.05$ compared to APZ (0.1 mg/kg) and $^{d}P < 0.05$ compared to APZ (1 mg/kg) [One-way ANOVA followed by Student Newman-keuls test].

5.3.14 APZ inhibits the expression of Caspase-3

The effects of repeated APZ (0.1, 1.0 and 10mg/kg) and PAX-10mg/kg on the levels of expression of caspase-3 in different brain regions are illustrated in Fig-5.13. Statistical analysis of data showed significant differences among groups in the level of expression of caspase-3 in PFC [$F(5, 12) - 47.3$, $P < 0.05$] and AMY [$F(5, 12) - 45.6$, $P < 0.05$]. Post-hoc the analysis revealed that SRS significantly increased the expression of the enzyme caspase-3 in both PFC and AMY compared to control. Repeated treatment of APZ in the dose of 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.

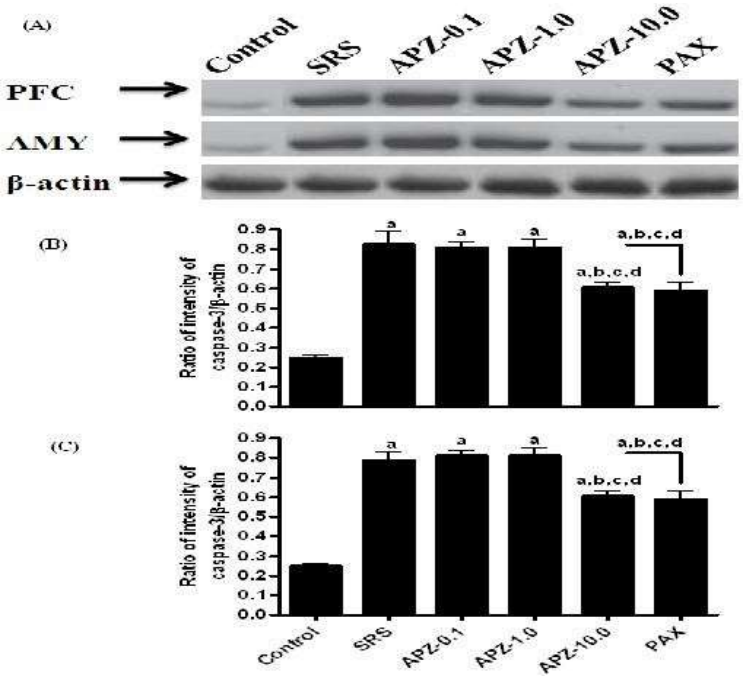

Fig 5.13: The effect of APZ (0.1, 1 and 10 mg/kg) and PAX on SRS induced changes in the expression of caspase-3 in PFC and AMY. (A) Indicates the blot representations of caspase-3 in PFC and AMY. (B) and (C) is the histogram data expressed as the ratio of the relative intensity of levels of caspase3 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, $^{c}P < 0.05$ compared to APZ (0.1 mg/kg), $^{d}P < 0.05$ compared to APZ (1 mg/kg) [One- way ANOVA followed by Student Newman-keuls test].

5.4 Discussion

APZ shows anti-PTSD-like effects in the stress re-stress model of PTSD in rats. This is the first study showing the anti-PTSD potential of APZ in pre-clinical studies. Also, the anti-PTSD effects of APZ are shown to be due to modulation of plasma corticosterone as well as enhancement of neuronal protective factors. SRS model of PTSD showed an enhancement in anxiety-like behaviour in EPM. The SRS showed a decrease in open time spent, open arm entries, fecal pellets, immobility time. Similar effects were found in a previous study with the SRS model of PTSD in rats used to evaluate OLZ (Reddy et al., 2018). These SRS-induced effects were decreased by APZ at a dose of 10mg/kg and also by PAX at a dose of 10mg/kg, indicating their ability to decrease anxiety-like symptoms. Similar results were produced by APZ in EPM in a genetic rat model with mild depression (Russo et al., 2013). Other experiments also showed the anxiolytic-like properties of PAX at a dose of 10mg/kg in EPM (Drapier et al., 2007).

SRS also showed a decrease in the number of entries in trial-1 and trail-2 and this was mitigated by APZ at a dose of 10mg/kg. PAX also showed an enhancement in the number of entries in both trails, indicating an increase in curiosity behaviour. Further, the coping behaviour and spatial memory were reduced by SRS and this was mitigated by APZ 10mg/kg and also PAX- 10mg/kg. The ability of APZ to mitigate spatial memory was reported in a rat model of absence epilepsy and mild-depression comorbidity (Russo et al., 2013). Similarly, at 10mg/kg dose PAX mitigated the disturbed spatial learning and memory in depressed rats (Han et al., 2015).

The plasma corticosterone was decreased by SRS-induced PTSD, indicating the disturbance in the HPA axis, which leads to hypocorticosteronemia (Reddy et al., 2018). This is a primary symptom of PTSD and this was enhanced by APZ at 10mg/kg. However, PAX did not show any changes in plasma corticosterone, indicating its lack of HPA axis modulation (Krishnamurthy et al., 2013).

The SRS showed significant changes in the 5HT levels in the rat brain regions like AMY but not in PFC. But, it had no effects on DA levels in any of the two brain regions. The repeated treatment with APZ 10mg/kg and PAX 10mg/kg mitigated the SRS-induced alteration in 5HT levels in the AMY region.

Further, SRS showed a decrease of BDNF and CREB expressions in the PFC and AMY, indicating its degenerative effect on the nervous tissue, as found in previous studies (Reddy et al., 2018). The treatment with APZ 10mg/kg and PAX mitigated the decreased expression of these two proteins due to SRS. In a rat model of acute swim stress, APZ was found to enhance BDNF levels at a dose of 10mg/kg (Luoni et al., 2014b). p-ERK was also enhanced by SRS in both PFC and AMY. This enhanced expression of p-ERK was decreased by APZ 10mg/kg and also PAX. p-ERK is thought to promote homeostasis and even cell death based on stress. Caspase-3 is an apoptotic enzyme that promotes cell death. SRS enhanced caspase-3 levels and this was effectively mitigated by APZ at 10mg/kg dose and also by PAX. Similar results were found in the stress restress model of PTSD in rats wherein caspase-3 was enhanced in the PFC region (Zhang et al., 2016). APZ was also shown to decrease the expression of caspase-3 in both PFC and AMY at a dose of 10mg/kg. This ability of APZ was shown to be due to inhibition of TNF-α as well as enhanced expression of BDNF (Gil et al., 2018; Takahiro et al., 2008).

From the above discussion, the SRS model induced disturbances in cell regulating factors like BDNF, CREB, p-ERK and caspase-3. These disturbances were effectively mitigated by APZ at a dose of 10mg/kg and also by PAX at 10mg/kg. The changes brought about by SRS in terms of anxiety-like and cognitive symptoms were also significantly mitigated by APZ at the selected dose of 10mg and were comparable to that of PAX 10mg/kg.