2.1 Introduction

Aripiprazole (APZ) is an atypical antipsychotic drug used in the treatment of schizophrenia (Mello et al., 2008). It is also used as an augmentative for therapy of mood disorders like anxiety, depression and post-traumatic stress disorder (PTSD) (Ratajczak et al., 2013). Clinical studies have also shown the effectiveness of APZ in stress-related mood disorders like anxiety, depression and PTSD (Rush et al., 2006). Risperidone, another atypical antipsychotic, possesses significant anti-stress properties (Krishnamurthy et al., 2011). This anti-stress property is proposed to be due to its effects on the serotonergic system. Serotonin is one of the key regulators of stress.APZ having significant effects on the serotonergic system could possess anti-stress effects (Pollier et al., 2000). Unlike other antipsychotic drugs, it has a low propensity for extrapyramidal side effects (EPS), weight gain, sedation, and elevation in serum prolactin levels (Bhachech, 2012). Hence, it is relatively safe for use in the above disorders, which require prolonged treatment for months to a few years.

The stress responses elicited from two centrally interrelated pathways viz. hypothalamic-pituitary-adrenocortical (HPA) system and the sympathetic nervous system (SNS). Neurotransmitters like dopamine (DA) and serotonin (5HT) are also involved in the adaptation mechanism of stress responses (Pani et al., 2000). These neurotransmitters have a reciprocal modulation with the HPA axis in regulating stress response (Pani et al., 2000). The 5-HT1A and 5-HT2A receptor subtypes play a major role in the epidemiology of stress disorders and are predominant in the HIP and prefrontal cortex (PFC) (Pani et al., 2000). Similarly, D2 receptors in the mesolimbic system contribute to plasticity during stress and help in relieving depression (Jordan et al., 2002). Conducive to it APZ elicits its response on both types of receptors. It is a partial agonist at 5HT1A receptors and an antagonist at 5-HT2A receptors (Jordan et al., 2002). Also, it is a partial agonist at D2 receptors. Many

studies reported that atypical antipsychotic drugs, including APZ, produce their effects through their action on 5-HT1A receptors at low doses and on D2 receptors in higher doses [16-18]. The partial agonistic activity at 5HT1A receptors leads to improvement from anxiety, depression, and cognitive dysfunction (Meltzer and McGurk, 1999). while binding to the D2 receptors improves antipsychotic symptoms (Seeman, 2002). Therefore, it is possible that APZ acting through 5HT1A and D2 receptors can bring changes in the serotonergic and dopamine transmissions and thereby modulate neurochemical pathways of stress. Antipsychotics show significant dose-dependent effects (Seeman, 2002). When given in low doses, they preferably act on one distinct type of receptor to elicit their response. Hence, dose variation may provide additional information on their mode of action and specific physiological changes. Previous studies also evaluated the effect of APZ in brain monoamines (de Bartolomeis et al., 2015). So, the effects of APZ on monoamines in the stressed brain would also help understand the neurochemical mechanism of the drug.

The present study evaluates the anti-stress effects of repeated APZ treatment and ensuing dose differences in the CRS model in rats. Parameters like gastric ulcer, plasma corticosterone (CORT) and norepinephrine (NE) levels are measured as indices of stress, the HPA axis, and the SNS, respectively. Further, the effect of the APZ on the stress-induced changes in the monoaminergic system was measured in terms of 5-HT, its metabolite 5-hydroxy indole acetic acid (5-HIAA), dopamine (DA) and its metabolite; 3, 4 dihydroxy phenylacetic acid (DOPAC) in brain regions like HIP, PFC and striatum (STR). To check for any possible extrapyramidal side effects (EPS), cataleptic behaviour was also evaluated.

2.2 Material and methods

2.2.1 Animals

Charles Foster strain rats (average weight 220-260) were procured from the central animal house, BHU. All the animals were housed and treated 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). The experimental procedures were prior approved by the Institutional animal ethical committee, Banaras Hindu University (Ref No. Dean/10-11/148). The rats were grouped as the control, stress, doses of 0.1mg, 1mg and 10mg/kg body weights of APZ to be administered.

2.2.2 Drugs and chemicals

APZ was procured from Sigma (St. Louis, MO, USA). All other chemicals and reagents of HPLC were of analytical grade and procured from local suppliers.

2.2.3 Drug treatment

Animals were orally pretreated with 0.5% carboxymethylcellulose (CMC) suspensions of either Aripiprazole (APZ) for 21 days with fixed doses of 0.1, 1.0 and 10.0 mg/kg for three different treatment groups. Control and sham groups were administered with just 0.5% CMC. Dose ranges were selected based upon related antipsychotic doses (Eren et al., 2007).

2.2.4 Cold restraint stress (CRS)

The CRS procedure was followed the same as earlier mentioned (chapter-1). After the experimental schedule at the last day of the experiment, rats were decapitated and heparinized blood was collected and brain samples were stored at -80^{o}c until further experimentation (Pacchioni et al., 2002).

2.2.5 Evaluation of Catalepsy behaviour

This behavioural parameter is screened to evaluate the extrapyramidal side effects of antipsychotic drugs (Karl et al., 2006). Since different doses of drugs are used, the presence of catalepsy behaviour indicates if the drug in the doses used is effective in the absence of extrapyramidal side effects. Catalepsy is defined as the acceptance and retention of abnormal posture and was measured employing the bar test. The bar test was carried by gently removing rats from their home cage and placing their forepaws on a horizontal bar, fixed at the height of 10 cm above the working surface. The length of time during which the animal retained in this position was recorded by measuring the time from the placement of the rat until removal of one of its forepaws (mean of three consecutive trials; cut-off time =60s). All the groups were tested on the 21st day.

2.2.6 Evaluation of Ulcer Index

The stomach was cut through greater curvature, and the ulcer index was calculated by following standard protocol by a blind observer. The ulcer index is the function of the severity of stress. The drug's ability to mitigate stress can be observed by the reduction in ulcer index (Cho and Ogle, 1979).

2.2.7 Estimation of plasma corticosterone and norepinephrine by HPLC

The levels of plasma corticosterone were measured by HPLC using a UV detector. While the levels of plasma norepinephrine (NE) were estimated by HPLC connected with an electrochemical detector (ECD) (Krishnamurthy et al., 2011).

2.2.8 Estimation of Serotonin, Dopamine, norepinephrine (NE) and their Metabolites by HPLC

The brains were removed after decapitation and microdissected as soon as possible on glass plates over ice into five regions: the prefrontal cortex (PFC), hippocampus (HIP), amygdala (AMY) and hypothalamus (Palkovits et al., 1976). The levels of 5-HT, DA and their metabolites were estimated using HPLC/ECD (Palkovits et al., 1976). In brief, the brain tissue samples were homogenized in 0.17 M perchloric acid by a Polytron homogenizer. Homogenates were then centrifuged at 33,000g (BiofugeStratos, Heaureas, Germany) at 4°C. Twenty microliters of supernatant were injected via HPLC Mobile phase consisted of methanol: water (70:30) pump (Model 515, isocratic pump, Waters, Milford, MA, USA) into a column (Spherisorb, RP C18, 5 lm particle size, 4.6 mm i.d. 9250 mm at 30°C) connected to an ECD (Model 2465, Waters, Milford, MA, USA) at a potential of 0.8V with a glassy carbon working electrode Vs. Ag/AgCl reference electrode. Mobile phase consisted of 32mM citric acid, 12.5mM disodium hydrogen orthophosphate, 1.4mM sodium octyl sulfonate, 0.05mM EDTA and 16% (v/v) methanol (pH 4.2) at a flow rate of 1.2ml/min. Quantification was done by comparing the peak heights of the samples to the corresponding standard curve. Two ranges of standard curves, i.e., 10-100 and 100-1,000ng/ml, were used depending upon the abundance of monoamines in respective brain regions. A constant amount (25ng/ml) of DHBA was added to the tissue samples to calculate recovery. The protein content was estimated to quantify the neurotransmitters in terms of a fixed weight of protein (Lowry et al., 1951).

2.2.9 Statistics for Data Analysis

The plasma CORT, NE and brain monoamines data were analyzed by using a Graph pad prism 5. Statistical analysis of data was done using one-way ANOVA with Newman-Keuls Post-hoc analysis. For correlation studies, Pearson's correlation analysis was used to correlate the changes in gastric ulcer, plasma corticosterone and brain monoamines.

2.3 Results

2.3.1 Effect of aripiprazole (APZ) on CRS-induced alteration in cataleptic behaviour

Fig.2.1 depicts the effect of repeated APZ treatment at doses 0.1, 1.0 and 10mg/kg on catalepsy behaviour. Statistical analysis by One-way ANOVA [F (4, 24) = 3.715, p>0.05] revealed significant differences in cataleptic behaviour among the groups. Post Hoc analysis showed that APZ did not show catalepsy behaviour at all doses tested

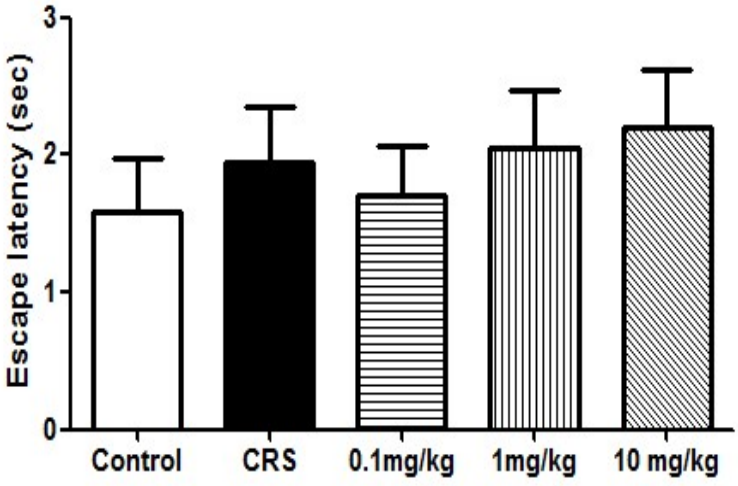

Fig-2.1 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on stress-induced changes in catalepsy behaviour on bar test. All the values are Mean ± SEM with n=5 [One-way ANOVA followed by Student Newman-keuls test].

2.3.2 Effect of aripiprazole (APZ) on CRS-induced alteration in ulcer index

Fig-2.2 shows the effect of repeated APZ treatment at doses 0.1, 1.0 and 10mg/kg on Stress in terms of ulcer index. Statistical analysis by One-way ANOVA revealed that there was significant interaction among groups [F (4, 24) = 84.21, p<0.05]. Post-hoc analysis showed that stress significantly increased the ulcer index compared to control. Repeated treatment with APZ at doses of 0.1, 1.0 and 10mg/kg significantly reduced ulcer index compared to the Stress group.

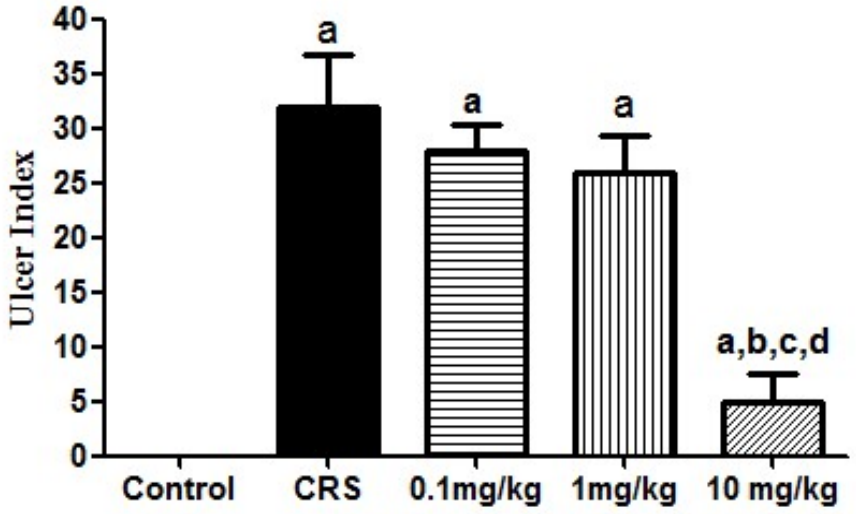

Fig 2.2 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on stress-induced changes in gastric ulcers. All the values are Mean ± SEM with n=5. [a]P<0.05 compared to control, [b]P<0.05 compared to CRS, [c]P<0.05 compared to APZ (0.1 mg/kg) and [d]P<0.05 compared to APZ (1 mg/kg) [One-way ANOVA followed by Student Newman-keuls test].

2.3.3 APZ alters plasma CORT and NE in Stressed animal

The effect of repeated APZ treatment at doses 0.1, 1.0 and 10mg/kg on plasma CORT and NE is depicted in **Fig-2.3 & 2.4,** respectively. Statistical analysis showed that there was a significant difference in the concentration of plasma CORT [F (4, 24) = 11.18, $p<0.05$] and NE [F (4, 24) = 4.445, $p<0.05$] among groups. *Post-hoc* analysis by Newman–Keuls test revealed that stress significantly increased plasma CORT and NE levels. Plasma corticosterone was significantly decreased by APZ treatment at all doses. While the plasma NE levels were significantly decreased at 1.0 mg/kg of APZ dose compared to stress. However, other doses did not show any effect on stress-induced changes in plasma NE levels.

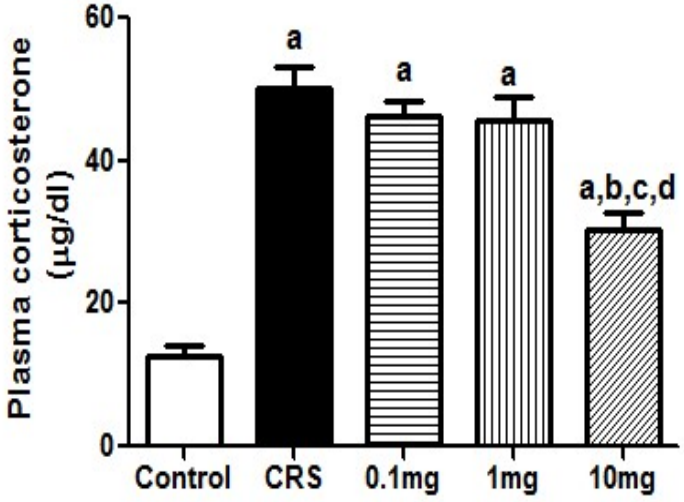

Fig-2.3 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on stress-induced changes in plasma corticosterone. All the values are Mean ± SEM with n=5. [a]P<0.05 compared to control, [b]P<0.05 compared to CRS, [c]P<0.05 compared to APZ (0.1 mg/kg) and [d]P<0.05 compared to APZ (1 mg/kg) [One-way ANOVA followed by Student Newman-keuls test].

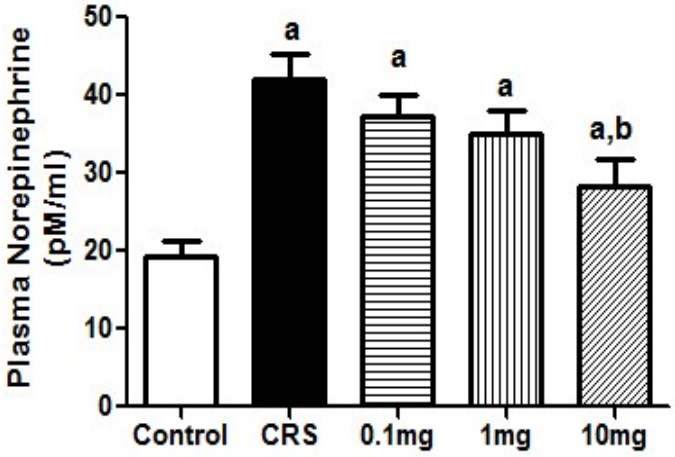

Fig-2.4 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on stress-induced changes in plasma norepinephrine. All the values are Mean ± SEM with n=5. [a]P<0.05 compared to control and [b]P<0.05 compared to CRS [One-way ANOVA followed by Student Newman-keuls test].

2.3.4 APZ selectively alters the level of 5-HT and its metabolites in the hippocampus, prefrontal cortex, Amygdala and hypothalamus

5HT: Repeated treatment with APZ (0.1, 1.0 and 10 mg/kg) on 5-HT levels among brain regions is shown in **Fig-2.5**. One-way ANOVA shows that, there was significant differences among groups in the 5-HT levels in PFC [F (4, 24) = 7.304, $p>0.05$, HIP [F (4, 24) = 18.5, $p<0.05$], AMY [F (4, 30) = 6.305, $p<0.05$], HYP [F (4, 24) = 4.18, $p>0.05$. Post-hoc analysis by Newman-Keuls showed that Stress significantly increased 5-HT levels in all regions compared to control. Repeated doses of APZ also showed a decline in levels of 5-HT at all doses in PFC except in 1mg/kg dose. In the HIP, it showed a decline in stress-induced 5HT levels only in 10mg/kg dose but remained elevated in other doses. While in the AMY, a decline in 5HT levels compared to stress is seen at doses of 0.1 and 10mg/kg but not at 1mg/kg. Similarly, in HYP, repeated treatment in APZ mitigated 5HT levels compared to stress in doses of 0.1 and 1mg/kg but not in 10mg/kg dose.

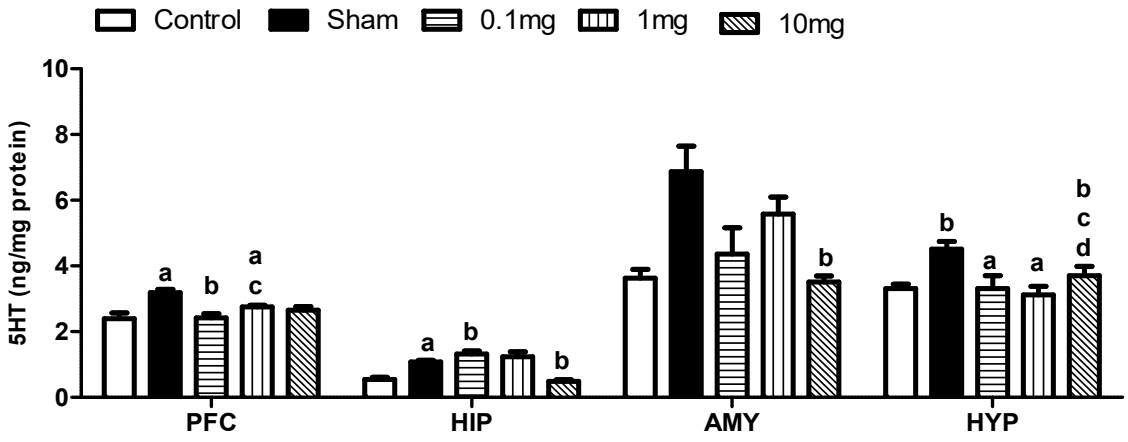

Fig 2.5. The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on brain 5HT levels in regions like PFC, HIP, AMY, HYP. All values are Mean ± SEM with n=6. $^{a}p<0.05$ compared to control, $^{b}p<0.05$ compared to Stress, $^{c}p<0.05$ compared to APZ (0.1 mg/kg) and $^{d}p<0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

5HIAA: Fig-2.6 shows 5-HIAA levels in different brain regions in cold restraint rats after repeated treatment with APZ. One-way ANOVA analysis indicates significant differences in the 5-HIAA levels PFC [F (4, 24) = 9.037, $p<0.05$]; AMY [F (4, 24) = 5.3, $p>0.05$]; HIP [F (4, 30) = 2.879, $p>0.05$] and HYP [F (4, 30) = 10.08, $p<0.05$]. Post-hoc analysis showed that Stress decreased 5-HIAA levels in all the brain regions. However, repeated APZ treatment (APZ; 0.1, 1 &10mg/kg) showed dose and region-based effects. In PFC, APZ increased the 5HIAA at 0.1mg doses but not in 1 and 10mg/kg doses. In HIP the 5HIAA levels increased at doses of 0.1 and 1mg/kg but not at 10mg/kg. In the AMY, the 5HIAA levels increased at the dose of 1mg/kg dose alone. While in HYP, APZ could not reverse the stress-induced decline in 5HIAA in the first two lower doses but reversed it in the highest dose.

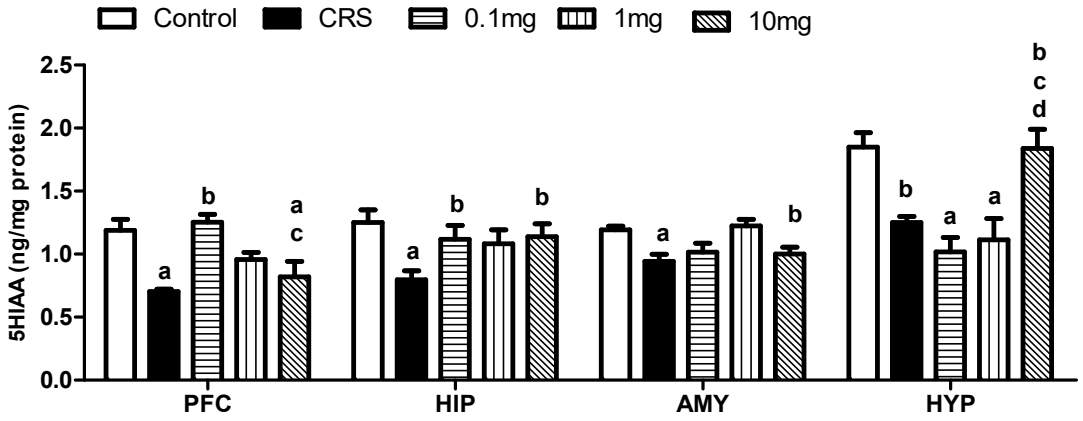

Fig-2.6 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on brain 5HIAA levels in regions like PFC, HIP, AMY, HYP. All values are Mean ± SEM with n=6. [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to Stress, [c]$p<0.05$ compared to APZ (0.1 mg/kg) and [d]$p<0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

5-HIAA/5-HT: Fig-2.7 represents the effect of repeated treatment of APZ on 5-HIAA/5-HT ratios in different brain regions. On analysis with one-way ANOVA there is significant interaction of treatment between groups; PFC [F (4, 24) = 9.565, $p<0.05$], HIP [F (4, 24) = 28.72, $p<0.05$] AMY [F (4, 24) = 16.29, $p<0.05$] and HYP [F (4, 24) = 8.637, $p<0.05$]. Further, Post-hoc analysis showed that the stress decreased the 5-HIAA/5-HT ratios in all the brain regions. This decrease in 5-HIAA/5-HT was reversed by APZ administration in varying doses at different brain regions. APZ mitigated the CRS-induced decline in 5-HIAA/5-HT in PFC at doses of 0.1 & 10mg/kg. In the HIP, this effect was shown at the only APZ dose of 10mg//kg. In AMY, APZ showed a reversal of the stress-induced decline in 5-HIAA/5-HT at all doses. In HYP, the reversal was observed at only 10mg/kg dose.

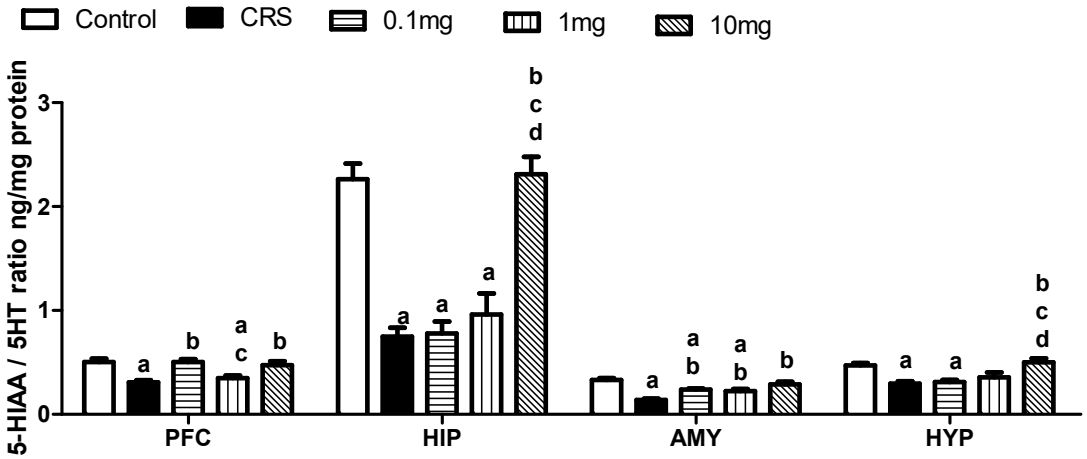

Fig-2.7 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on brain 5HIAA/5HT levels in regions like PFC, HIP, AMY, HYP. All values are Mean ± SEM with n=6. [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to Stress, [c]$p<0.05$ compared to APZ (0.1 mg/kg) and [d]$p<0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

2.3.5 APZ selectively alters the level of DA and its metabolite in the prefrontal cortex, hippocampus Amygdala and hypothalamus

The effect of repeated treatment of APZ (0.1, 1.0 and 10 mg/kg) on DA levels in different brain regions in stress is depicted in **Fig-2.8**. Analysis of data by One-way ANOVA showed that there was significant differences in the DA levels in PFC [$F(4, 24) = 8.907$, $p<0.05$], HIP [$F(4, 24) = 9.744$, $p>0.05$], AMY [$F(4, 24) = 4.272$, $p>0.05$]and HYP [$F(4, 24) = 9.917$, $p<0.05$. Post-hoc analysis showed that Stress significantly increased the DA levels in all the brain regions. Repeated APZ (APZ; 0.1 mg/kg and 1.0 mg/kg) treatment mitigated the stress-induced increase in DA levels at doses of 10mg/kg in PFC and AMY. While in the regions like HIP and HYP, the enhanced DA levels were reduced in doses of 0.1 and 1mg/kg doses, respectively.

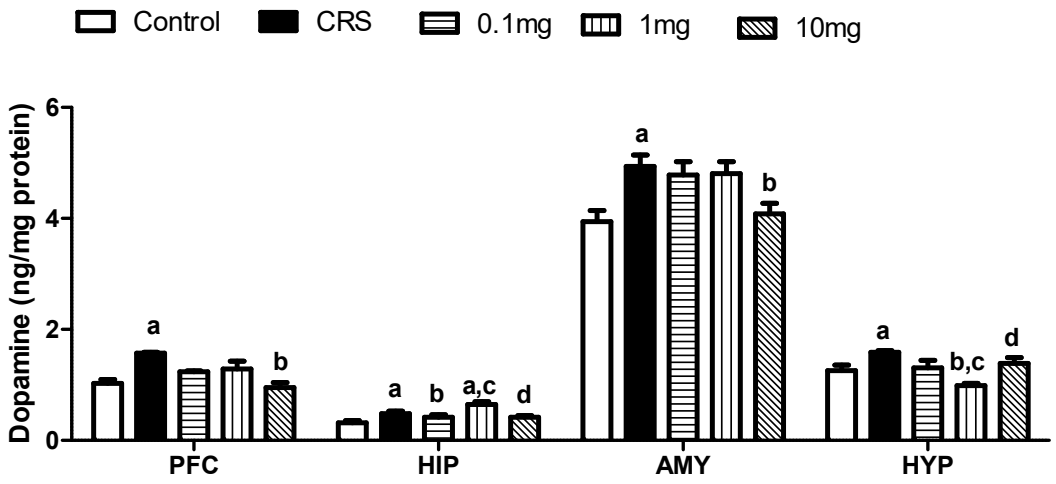

Fig-2.8. The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on brain dopamine levels in regions like PFC, HIP, AMY, HYP. All values are Mean ± SEM with n=6. $^{a}p<0.05$ compared to control, $^{b}p<0.05$ compared to Stress, $^{c}p<0.05$ compared to APZ (0.1 mg/kg) and $^{d}p<0.05$ compared to APZ (1mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

The effect on DOPAC levels in different brain regions APZ (0.1, 1 and 10 mg/kg) in stress is depicted in **Fig-2.9.** One-way ANOVA revealed significant differences among groups in the DOPAC levels in PFC [F (4, 24) = 12.89, $p<0.05$]; HIP [F (4, 24) = 12.97, $p<0.05$], AMY [F (4, 24) = 4.174 $p<0.05$] HYP= 5.336. Post-hoc analysis showed that stress enhanced DOPAC levels in all the regions. In PFC, this change was reversed by all doses of APZ administered. In AMY and HYP, this change was mitigated in doses of 10 and 1 mg/kg doses, respectively. Whereas, in the HIP, the elevation of DOPAC by stress was mitigated by APZ only at doses of 0.1 and 10mg/kg.

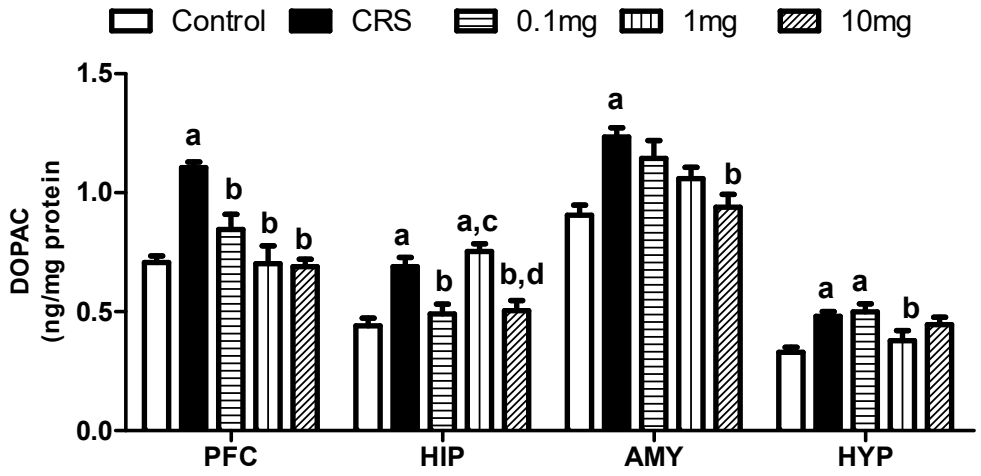

Fig-2.9 Effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on brain DOPAC levels in regions like PFC, HIP, AMY, HYP. All values are Mean ± SEM with n=6. $^{a}p<0.05$ compared to control, $^{b}p<0.05$ compared to Stress, $^{c}p<0.05$ compared to APZ (0.1mg/kg) and $^{d}p<0.05$ compared to APZ (1mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

The effect of repeated treatment of APZ (0.1, 1 and 10mg/kg) on DOPAC/DA ratios in different brain regions in Stress is illustrated in Fig-2.10. Statistical analysis by One-way

ANOVA showed that there was a significant interaction of treatment with respect to DOPAC/DA ratios in PFC [F (4, 24) = 7.338, $p<0.05$], HIP [F (4, 24) = 19.61, $p<0.05$], AMY [F (4, 24) = 8.903, $p<0.05$] and HYP [F (4, 24) = 4.092, $p<0.05$] among groups. Post-hoc analysis indicated that stress significantly increased DOPAC/DA ratios in all brain regions. The stress-induced rise in DOPAC/DA levels was mitigated by all the doses of APZ administered in AMY. In PFC, it was decreased in doses of 1 and 10mg/kg doses. In the HIP, the levels were reduced compared to the stress at doses of 0.1 and 1mg/kg doses. Whereas in HYP, there was a decrease at a dose of 10mg/kg only.

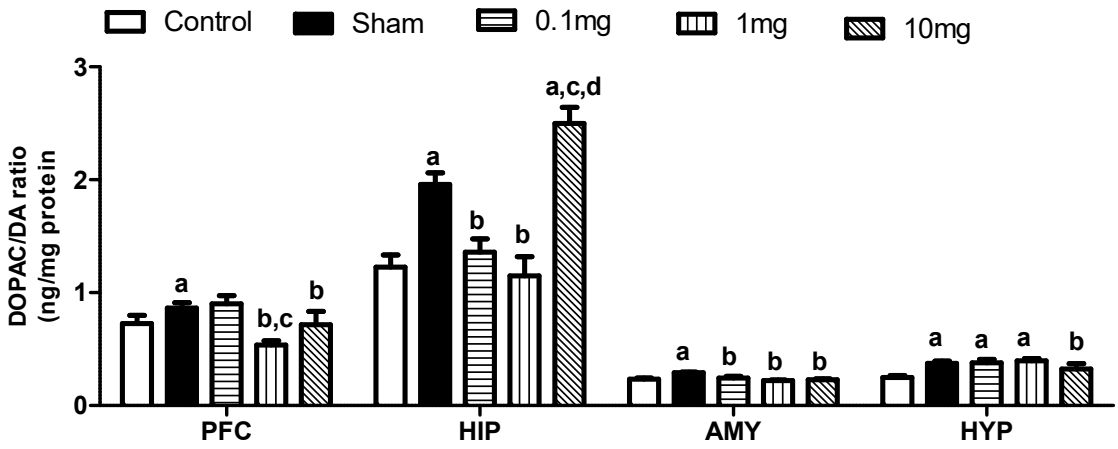

Fig-2.10 The effect of repeated treatment of APZ (0.1, 1.0 and 10mg/kg) on brain DOPAC/DA levels in regions like PFC, HIP, AMY, HYP. All values are Mean ± SEM with n=6. [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to Stress, [c]$p<0.05$ compared to APZ (0.1 mg/kg) and [d]$p<0.05$ compared to APZ (1 mg/kg). [One-way ANOVA followed by Student Newman-keuls test].

2.3.6 Significant Correlation exists between gastric ulcer, plasma corticosterone and monoamines in discrete brain regions

Correlation analysis between markers of stress and brain monoamines is depicted in **Fig-2.11.** Fig. 2.11 (A) illustrates the correlation between the two markers of stress viz. Gastric ulcer and plasma corticosterone. A significant positive correlation (A; r^2= 0.5597, Pearson's r = 0.7482) was observed between gastric ulcer and plasma corticosterone. While considering correlation analysis between plasma corticosterone and serotonin in discrete brain regions, significant correlation was observed Fig-2.11 (BCDE). In PFC (B; positive; r^2= 0.26, Pearson's r=0.8841), in HIP (C; positive; r^2=0.2070, Pearson's r=0.4550), AMY (D; positive; r^2= 0.7816, Pearson's r=0.8841) and in HYP (E; Positive; r^2= 0.2073, Pearson's r=0.4553). Correlation analysis between plasma corticosterone and dopamine is shown in Fig 2.11 (FGHI). There is a significant correlation between plasma corticosterone and dopamine in PFC (F; positive; r^2= 0.4644, Pearson's r= 0.6815), and in HIP (G; positive; r^2= 0.2617, Pearson's r= 0.5116), in AMY (G; positive; r^2= 0.1073, Pearson's r= 0.3275) and in HYP (G; positive; r^2= 0.01648, Pearson's r= 0.1284). Thus, monoamines serotonin and dopamine showed a significant correlation with plasma corticosterone under the conditions of stress.

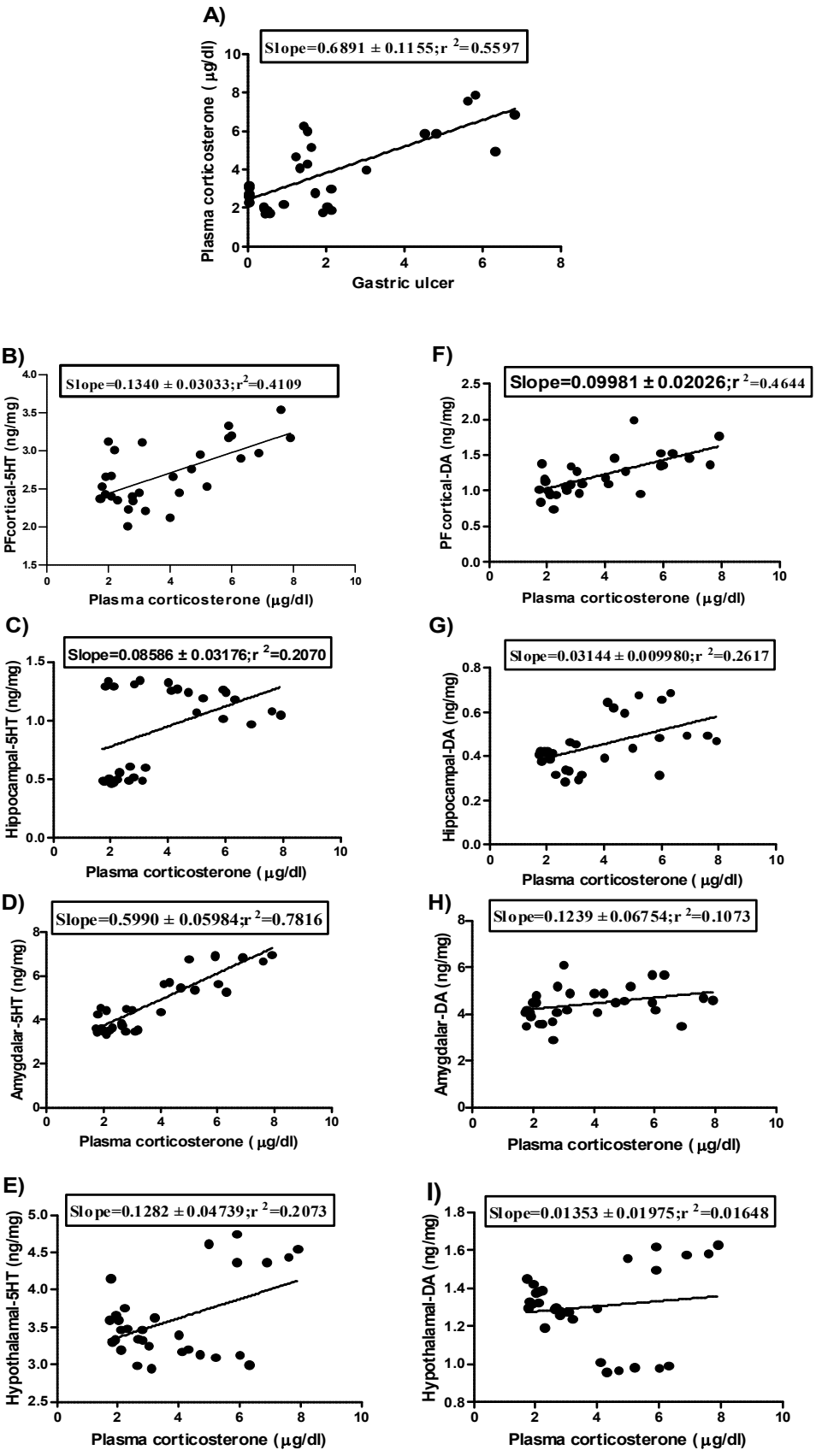

Fig. 2.11. The correlation between Plasma corticosterone, 5HT and DA levels. [Pearson's correlation analysis at P <0.05].

1

2.4 Discussion

The present study was performed to examine the anti-stress effects of APZ in the cold restraint stress model of rats. The main findings of the study are the attenuation of stress-induced elevation of gastric ulcers, plasma corticosterone, and nor-epinephrine by repeated APZ treatment. Another intriguing discovery is, APZ showed prominent mitigation of the stress-induced perturbations of brain monoamines in stress-sensitive brain regions.

Gastric ulcer is one of the key markers of stress in animal models. Both cold immobilization stress and restraint stress models of rats have been shown to cause significant gastric ulcers (Koo et al., 1986). In the present study, the gastric ulcer induced by restraint stress was calculated as the ulcer index. Repeated treatment with APZ in all the doses of 0.1, 1 and 10mg showed a significant decrease in ulcer index. Stimulation of the HPA axis by stress may be responsible for gastric ulceration. Especially the para-ventricular nucleus, which is the key stress-sensitive region within the hypothalamus, may be involved in this response (Herman et al., 2008). The PVN on stimulation by stress induces gastric ulcers and triggers the release of corticotrophin-releasing hormone (CRH) into the systemic circulation (Cook, 2004). This CRH secretion leads to rising in plasma corticosterone due to the stimulation of the adrenal cortex. This stimulation of the adrenal cortex also raises plasma norepinephrine levels (Cook, 2004). This response of CRH secretion by the HPA axis during stress is considered to be a homeostatic mechanism of the body to manage stress. However, when there is an insufficient response to HPA axis signals, it can derange the physiological homeostasis leading to ulcers. The possible anti-stress in terms of gastric ulcer by APZ could be due to the combined effects of central and peripheral pathways. In a recent study of ethanol-induced gastric ulcer activity, APZ showed a significant reduction in ulcer formation. It was found that APZ showed anti-ulcer effects due to its anti-secretory, antioxidant, anti-inflammatory actions and also by its ability to restore the depleted gastric serotonin levels (Al

Asmari et al., 2014).

Catalepsy behaviour is indicative of extrapyramidal side effects, which result due to the binding of D2 receptors by antipsychotics (Petrovic). This blockade seems to occur in those antipsychotics, which bind to D2 receptors besides 5HT receptors, which are their primary receptors as their target for the action (Tuunainen and Wahlbeck, 2000). Atypical antipsychotics show antipsychotic effects at a binding threshold of 65% and EPS at a binding threshold of 80%. In the present study, APZ did not show any EPS at all the doses tested. This indicates that it has a low propensity to cause EPS while being effective in stress. Plasma corticosterone is one of the important markers of stress induced by the alteration of the glucocorticoid system through the HPA axis (Miura et al., 1993). In stress plasma, corticosterone levels rise and drugs, which are effective in stress, seem to reverse this effect (Krishnamurthy et al., 2013). Plasma corticosterone was significantly reduced on treatment with APZ in CRS-subjected rats. Stress significantly elevates plasma glucocorticoids (GCs) production (Miura et al., 1993), and plasma norepinephrine is increased due to psychosocial stress. This rise in plasma NE is minimized by repeated administration of APZ, indicative of its anti-stress action.

Under the conditions of stress, aberrations of the major brain monoamines, viz. dopamine and serotonin, are observed (Krishnamurthy et al., 2013). Monoamines like 5HT and DA are thought to be clearly elevated in stress. This elevation in the monoamines during stress is believed to be due to the increased activity of these neurotransmitters. This enhanced activity is thought to be a mechanism to counter stress-induced derangement of homeostasis (Rueter et al., 1997). In our study, both of the monoamines showed an elevation in the brain regions due to stress. The repeated treatment showed a decline in elevated serotonin in all the regions like PFC, AMY, HYP, and HIP but had dose variations. In PFC, AMY, and HYP, the reversal of elevation in 5HT was seen at all doses, but in the HIP, it was observed at only

10mg/kg dose. During stress, serotonin transmission is higher as a counter mechanism to stress. 5HT released from neurons due to excess firing is taken back into the synaptic cleft. In the synaptic cleft, some amounts are taken up into storage vesicles, while the remaining is metabolized by MAO to form 5HIAA. So during stress, since there is enhanced serotonergic transmission, there should have been a high amount of 5HIAA formed in the brain regions (Shannon et al., 1986). However, in our study, the 5HIAA levels declined significantly in comparison to the control. This might be due to a decrease in 5HT metabolism by protein tribulin, which is increased in times of immobilization stress (Bhattacharya et al., 1988). The decline in 5HIAA levels was reversed in all the regions except at doses of 0.1 and 1mg/kg dose in HYP. The ratio of 5HIAA to 5HT is an indicator of serotonergic activity in the brain. Since there is an elevation in 5HT but a decrease in 5HIAA levels, correspondingly, there is a decline in 5HIAA/5HT ratio due to stress in all the brain regions. This effect was reversed by APZ administration in all the regions dose specifically. In the HIP, the reversal in the stress-induced decline in the 5HIAA/ 5HT ratio was seen in all the doses. However, in PFC and HIP, this reversal was seen only at doses of 0.1, 10 mg/kg doses and 1, 10mg /kg doses in HYP. The amygdalar 5HIAA/5HT ratio was reversed at the highest dose of 10mg/kg. Another neurotransmitter, DA, showed elevated levels in all the regions tested. Several studies also sustain the view that not only stressful events but even mild environmental changes which can evoke emotional arousal are accompanied by increased DA extracellular concentrations. This rise in DA is partly due to circulating corticosteroids. The glucocorticoids have stimulant effects on dopaminergic transmission (Piazza et al., 1996). When the secretion of corticosterone is decreased, there is inhibition of the release of dopamine. Repeated treatment with APZ reversed stress-induced elevation of DA. Thus it can be inferred that reduction in the excessive rise of corticosterone levels by APZ in stress conditions leads to attenuation of elevated dopaminergic transmission (Rougé-Pont et al.,

1998). So, APZ, which has reduced the corticosterone levels, could have also mitigated DA concentrations in the brain regions mentioned above. In another study, it was reported that APZ decreased the DA, which was elevated by stress (Oshibuchi et al., 2009). It also augments the selective serotonin reuptake inhibitors (SRRI's), which are responsible for the reuptake of serotonin. Further, the DA metabolite DOPAC and DOPAC: DA ratio, which is indicative of DA turnover, is elevated in all the brain regions due to stress. Repeated treatment with APZ showed alleviation of DOPAC in all the doses in PF and HIP but not in AMY and HYP. On the other hand, APZ at all the doses tested showed the alleviation of DOPAC/DA in all the brain regions. So, in the above study, treatment with APZ seems to modulate stress pathways in such a way that plasma corticosterone levels are ameliorated and, thereby, brain DA levels are also mitigated. Hence, APZ appears to counter stress-induced homeostatic derangement in terms of monoamines in the different brain regions. Correlation statistics of the peptic ulcer, plasma corticosterone, and monoamines further indicate this relative stress modulating effect. A significant correlation exists between gastric ulcers and plasma corticosterone. This is shown in many previous studies that gastric ulcer and rise in plasma corticosterone levels are prime markers of stress (Filaretova and Bagaeva, 2016). There is also a significant correlation between plasma corticosterone against dopamine and serotonin levels in all brain regions. This indicates the changes in brain monoamines were concurrent to changes in stress markers like gastric ulcer and plasma corticosterone.

Thus, the study illustrates that APZ has anti-stress effects, as seen by the decrease in gastric ulcers, plasma corticosterone, and NE levels. Also, the changes in dopamine and serotonin levels in the brain regions due to stress are mitigated as an indication of a decline in stress susceptibility after treatment with APZ.