Effect of Sarsasapogenin in murine model of Post-Traumatic Stress Disorder, The Possible Role of Adenosine

Hurmat Malik[1], Gowhar Ali[2], Muhammad Usman[1], Mehreen Arif [1], Zainab Ahmad[1], Muhammad Sona khan[1], Khalid Rauf[1*], Robert D. E. Sewell[3]

[1]Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan

[2]Department of Pharmacy, University of Peshawar, KP Pakistan, Pakistan

[3]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, UK

Abstract

Post-traumatic stress disorder (PTSD) is a chronic neuropsychiatric illness caused by extremely painful and traumatic experiences. PTSD symptoms include mood disorders and impaired cognition. Sarsasapogenin (Sas) is a steroidal sapogenin with a neuroprotective profile. The current study aimed to evaluate the potential of Sas in PTSD-induced anxiety and depression using single prolonged stress (SPS) model and associated changes in adenosine, hypoxanthine, and inosine levels in frontal cortex, hippocampus, and Striatum. After exposure to SPS, selected groups of mice were treated daily with sarsasapogenin (Sas) at doses of 20, 40, and 60 mg/kg or normal saline or fluoxetine for 7 days and were evaluated for depression and anxiety-like behavior using the tail suspension test (TST) and marble burying test (MBT), respectively. Following behavioral tests, the post-mortem Str, frontal cortex, and hippocampus were screened for changes in adenosine, hypoxanthine, and inosine levels. Sas treatment significantly ameliorated depression and anxiety-like behaviors in the SPS group. Sas restored adenosine levels in the frontal cortex and striatum at 40 and 60 mg/kg doses. In addition, inosine levels were disrupted in the frontal cortex and hippocampus which were restored by Sas at all doses in the frontal cortex and at 60 mg/kg in the hippocampus. However, no significant changes in hypoxanthine levels were observed in the frontal cortex, hippocampus, or striatum. The attenuation of behavioral despair and anxious behavior by Sas may involve the modulation of adenosinergic pathways. Taken together, these findings imply that Sas is a potential candidate for the treatment of PTSD-induced behavioral despair.

Keywords: PTSD, Adenosine, Depression, SPS, Anxiety

1 Introduction

PTSD is a mental disorder induced by traumatic events that threaten physical integrity. The key symptoms associated with PTSD include hyperarousal, intrusive memories, emotional distress, and avoidance [1]. During the COVID pandemic, the estimated prevalence of collective PTSD was 17.5% in the global population [2]. A more recent survey showed that the prevalence of PTSD among journalists in Pakistan's Khyber Pakhtunkhwa area is as high as 48.6% [3]. Anxiety and depression are the major comorbidities associated with PTSD [4]. These symptoms are accompanied by dysregulation of numerous neurotransmitter systems, neuromodulators and functional or structural abnormalities in certain brain areas like the hippocampus, frontal cortex, Striatum, and amygdala [5-7]. The frontal cortex is involved in executive function and regulates emotional components by exerting inhibitory control on the amygdala, which is the threat processing center. In PTSD, inhibitory control of the frontal cortex is diminished in the amygdala, resulting in a heightened fear response. Furthermore, the prefrontal cortex influences striatal activity to regulate motivated, habitual, and goal-directed behaviors [8, 9]. Notably, in patients with PTSD, the decline in inhibitory tone involves increased striatal activity in the response inhibition test, which is complemented by reduced striatal activity for responsiveness and reward processing [8, 10]. Moreover, the hippocampal volume is decreased in patients diagnosed with PTSD, which is associated with impaired cognition involving dopaminergic modulation [11]. Hippocampus is responsible for conscious memory processing and context-encoding during fear conditioning [12]. Adenosine modulates homeostasis and serves as a neuromodulator in the brain, as it exerts neuroprotective as well as neurodegenerative effects [13]. Adenosine levels are markedly increased during brain insults due to increased consumption of ATP to maintain cell viability, which causes an abnormally high adenosine levels [14, 15]. Adenosine and its metabolites play significant role in mood regulation and cognitive impairment [16, 17]. Owing to imbalance between multiple brain regions and neurochemical disruption in distinct brain regions, PTSD is presented with wide array of symptoms and comorbidities [18], which are addressed via psychotherapy and pharmacotherapy [19]. Although serotonin reuptake inhibitors (SSRIs) are used to treat PTSD, these drugs have tolerability issues [20]. Both treatment approaches are associated with 60% relapse rate [21, 22]. Therefore, there is a global surge for novel pharmacological entities with increase tolerability.

PTSD has been characterized almost purely in psychological terms, with only a few psychophysiological observations in biological literature. While psychological studies of PTSD are vital, they should be supplemented with research into the neurological and biochemical mechanisms implicated in PTSD for better understanding of the pathology and treatment targets. Recent research implies the promising role of adenosine in behavioral despair and anxiety and sleep-induction [17, 23], and epilepsy [24]. Moreover, the adenosine system can block discharge from the amygdala and locus coeruleus and has neuroprotective effects [25]. Adenosinergic receptor modulators have been scientifically documented to alleviate anxiety and obsessive compulsive disorders by targeting selected adenosine receptors like A1 or A2 [26-28]. Adenosine modulators have shown anxiolytic effect in specific marble burying models via its interaction with selected adenosine receptors [29-31]. Additionally A1 and A2A subtypes, conversely modulate inhibitory and excitatory tone simultaneously maintaining balance [32, 33]. Drastic decline in adenosine signaling results in multiple neuropsychiatric illnesses and neurodegenerative diseases including anxiety and depression [17]. Structural and functional abnormalities of A2 receptors have been implicated in development of major depression [34]. Stress has been found to be the one of the most influencers that leads to over production and elevation of adenosine signaling in brain that leads to synaptic plasticity [35]. Upregulation of $A_{2A}R$ in different brain regions with associated synaptic plasticity has been the hall mark of depression and many other neuropsychiatric illnesses [36]. In murine model, chronic stress has been associated with altered adenosine levels and changes in A_{2A} receptor density across the mesolimbic system [37]. Adenosine altered restoration by adenosine blockers has been reported to reverse chronic stress induced changes in hippocampus structures and functions and with restoration of corticosterone levels [17].

(Sas) is a steroidal sapogenin isolated from the rhizome of the Chinese herb, Anemarrhena asphodeloides Bunge. It has been found to improve cognition in aged rats by boosting the muscarinic receptor density [38]. Sas also has antidepressant-like characteristics, since it decreased immobility time in the FST [39]. Furthermore, Sas has been shown to reduce diabetes-induced memory loss, reduce neuroinflammation through the downregulation of the PAR-1 receptor [40] and provide neuroprotection by reducing Aβ peptide overproduction [41]. Despite having a strong anti-inflammatory profile and neuroprotective effects, Sas has not been

investigated for its pharmacological potential in PTSD; therefore, this study will investigate this possibility using a murine model of PTSD.

2 Material and methods

2.1 Animals

Male BALB/c mice (22-26 g) were procured from the National Institute of Health; Islamabad were used in the present studies. Mice were acclimatized to the experimental room one week prior to the experimentation and were kept in a controlled humidity (55 ± 15%), 12-12-hour dark/light cycle, and temperature (25 C) and were provided with water and food without any restriction. All the experimental protocols were approved by the Ethical Care Committee at the COMSATS University Islamabad, Abbottabad campus under registration number PHM.Eth/CS-M01-019-2901.

2.2 Materials

Sas (≥98% purity, CAS number 126-19-2) was procured from Haihang Industry Co Ltd., China. Fluoxetine was purchased from Aries Pharma, Peshawar. Adenosine, hypoxanthine, and inosine Acetonitrile, citrate buffer was procured from Sigma Aldrich USA.

2.2.1 Induction of SPS

In SPS, mice were subjected to immobilization for 2 hours in restrain tubes followed by 20 minutes forced swimming in clear glass tank (46 cm × 20 cm, 25±1 °C water temperature) filled with water up to two third of its height. Following that mice were recuperated for 15 minutes. Finally, they were subjected to ether vapors until they were unconscious. After recovering from unconsciousness, mice were returned to their home cages and left uninterrupted for 7 days to develop PTSD symptoms [42].

2.3 Experimental Protocol

After exposure to SPS, mice were randomly sorted into 6 groups. Group 1, (non-stressed +saline 10ml/kg), (Group 2, SPS+ saline), Group 3 (SPS+ Sas 20mg/kg), Group 4 (SPS+ Sas 40mg/kg), group 5 (SPS+ Sas 60mg/kg), group 6 (SPS + fluoxetine 10mg/kg). Sas and Fluoxetine were administered via IP route after exposure to SPS for 7 days. Following 7 days of treatment, behavioral tests were performed.

2.4 Behavioral Tests

2.4.1 TST

All behavioral tests were performed between 9.00 AM to 12:00PM. TST is widely employed to evaluate depression. The mouse's tail was firmly fastened to a wooden rod 50 cm above the surface in the TST chamber. To shield mice from visual distraction, a plexiglass enclosure was employed. The mice remained suspended for 5 minutes and their immobility time was taped using a video camera [43].Video was later analyzed by a trained blind observer.

2.4.2 Marble Burying Test

Propylene cages containing 5cm deep unscented, fresh mouse bedding were used. The surface of bedding was uniformly levelled by pressing another cage. It has the extra benefit of leaving a pattern of lines on the bedding for marble placement. Following this 20 glass toy marbles (5.2 g in weight, 5 mm diameter,) were placed gently on the bedding surface at equal distance from each other in 5 rows of 4 marbles. The marbles were thoroughly washed and cleaned with 70% alcohol to remove olfactory signatures. Mice were withdrawn from cages after 30 minutes of testing, and at least half to full covered of marbles in bedding were counted [44, 45].

2.4.3 Fecal Pellet output (FPO)

Rodents along with other species exhibit a stress response through alterations in colonic motor activity. In both humans and animals, increased fecal output is a reliable indicator of autonomic system alteration of colonic motility. The mice were kept in wooden chamber individually for 10 minutes and fecal pellet output was quantified and compared between groups [46].

2.5 Quantification of Adenosine, inosine, and Hypoxanthine

After completion of behavioral experiment, animals were euthanized and whole brains were extracted. Frontal cortex, hippocampus and Striatum were isolated from whole brains and were stored in Eppendorf tubes at -80 C to avoid degradation of adenosine and its metabolites. The isolated brain areas were then homogenized at 5000 rpm. in 0.2 percent perchloric acid with a Teflon glass homogenizer (Ultra-Turax®T-50). Following that, the samples were centrifuged at 4°C for 20 minutes at 12000 rpm (DLAB Scientific). 0.45 mm filter (CNW technologies) was used to filter the supernatant before being injected into an HPLC autosampler for analysis [47].

2.5.1 Chromatographic Conditions

Adenosine, inosine and hypoxanthine were quantified by HPLC using Waters Alliance 2690 separation module with an auto-sampler, UV detector, and PDA (USA) following [48] method. A C18 column (250× 4.6 mm, 5µm particle size) (Waters X Select® HSS Ireland) was used and

the mobile phase was prepared using 0.01M monobasic sodium phosphate and acetonitrile (95:5, v/v). Flow rate was.01/ml, temperature of the column was 35℃, and detection was carried out at 260 nm with isocratic elution in phosphate buffer [48].

2.5.2 Standard Preparation and Calibration Curve

Stock solutions of adenosine, hypoxanthine and inosine standards having a concentration of 1.0 mg/10ml were prepared. Several dilutions of 100-500 ng/mL were prepared from each stock solution. The samples were placed in the auto-sampler and 20µL sample injection was adjusted in the software (Empower TM). The peak area (y) was plotted against the concentration (x), using linear regression analysis to obtain the calibration curve. Unknown concentrations of the adenosine, inosine and hypoxanthine were quantified by comparing the respective peak areas [48].

2.6 Statistics:

Graph Pad Prism (version 8.2.1) was used to analyze the data and values were expressed as the mean ± SEM. Shapiro-Wilk test was applied on data for normal distribution. One-way ANOVA was applied with post-hoc Dunnett's test for analysis and $p<0.05$ was adopted as the threshold for significance.

3 Results

3.1 Effect of Sas on depression-like behavior in TST

In TST, mice exposed to SPS exhibited a significant increase in immobility time when compared to the non-stressed animals showing depression like behavior. All 3 doses of Sas and fluoxetine reduced the immobility time (Fig 1), indicating the amelioration of depressive behavior.

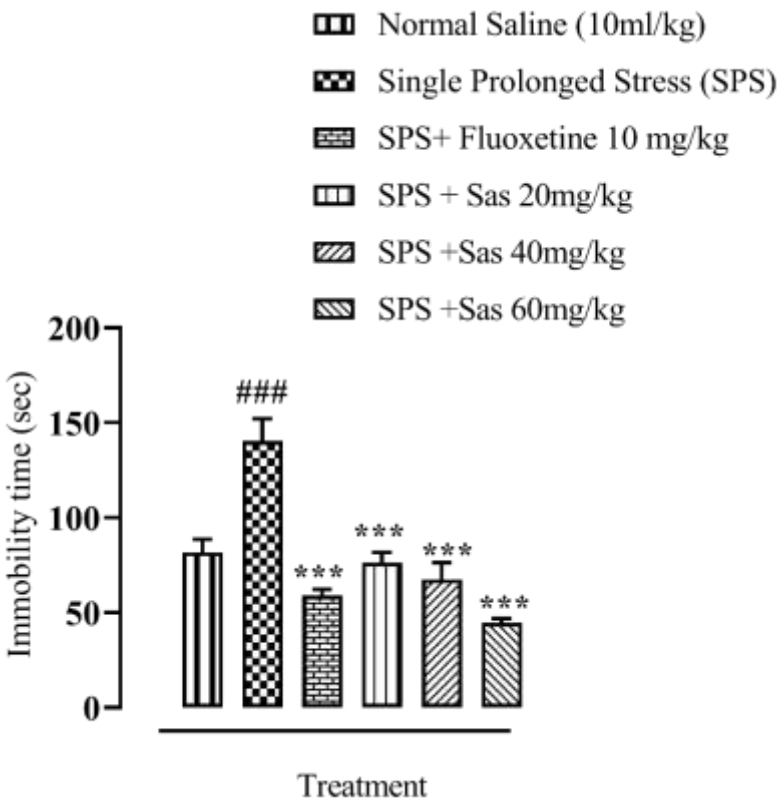

Figure 1. Effect of Sas or fluoxetine treatment on mouse behavioral despair induced by SPS in the FST. ### $p<0.001$ versus saline group, **$p<0.01$, ***$p<0.001$versus SPS group.

3.2 Effect of Sas on Anxiety like behavior in MBT

In MBT, the count of marbles buried by SPS group was significantly higher when compared to saline treated group which indicates anxiety in SPS exposed animals. Sas at highest dose and fluoxetine reduced the count of marbles buried in comparison with the SPS exposed group (Fig 2) hence indicating the anxiolytic effect.

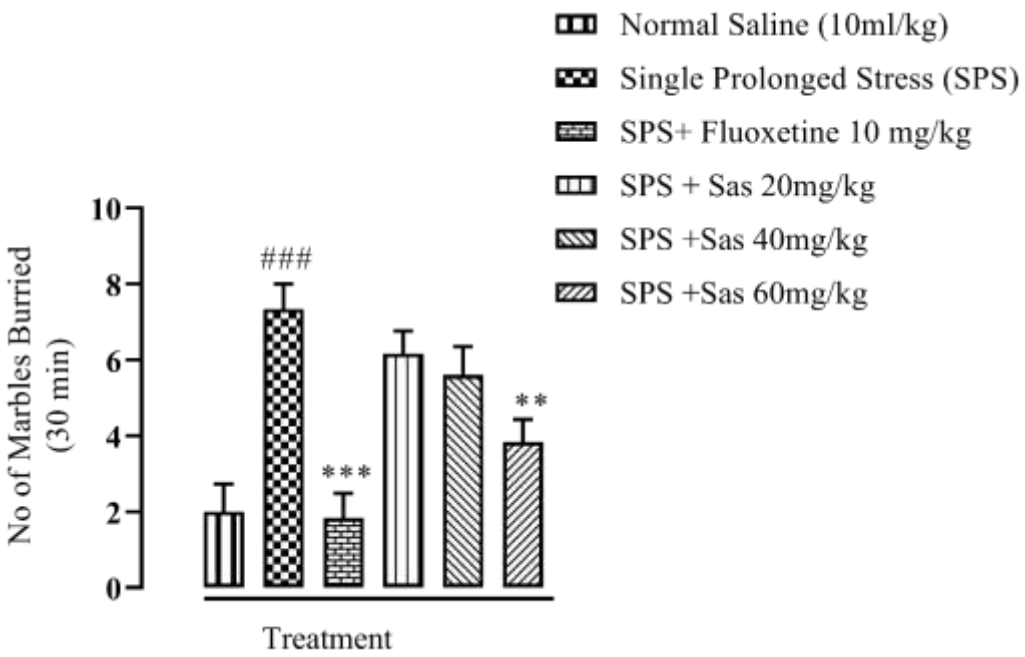

Figure 2. Effect of Sas or fluoxetine treatment on anxiety like behavior induced by SPS in the MBT. ### p<0.001 versus saline group, **p<0.01, ***p<0.001versus SPS group.

3.3 Effect of Sas on Fecal Pellet Output

No significant difference in fecal pellet output was observed among all groups, however, an increasing trend of FPO was observed in SPS group and a decline in FPO in fluoxetine and Sas groups.

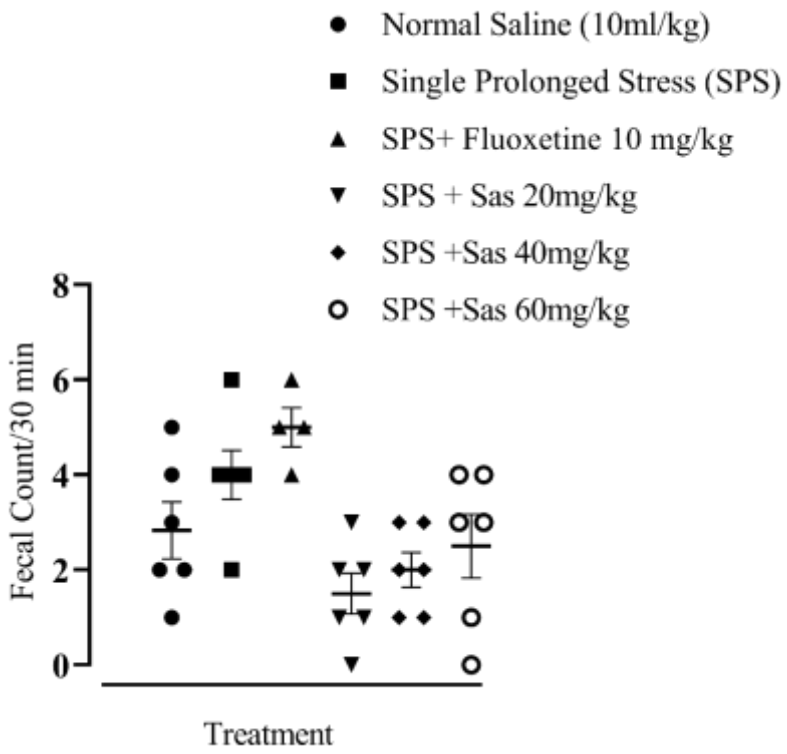

Figure 3: Effect of Sas or fluoxetine treatment on FPO. ### p<0.001 versus saline group, **p<0.01, ***p<0.001versus SPS group.

3.4 Effect of Sas on changes in frontal cortical adenosine, inosine, and hypoxanthine levels induced by SPS.

In SPS-exposed mice, adenosine levels were significantly increased in the frontal cortex which were significantly reduced by fluoxetine, and highest Sas dose (Fig 4A). Inosine levels were also increased in SPS exposed group which was attenuated by fluoxetine and Sas at all test doses (Fig 4B). However, no significant difference was observed in hypoxanthine levels (Fig 4C).

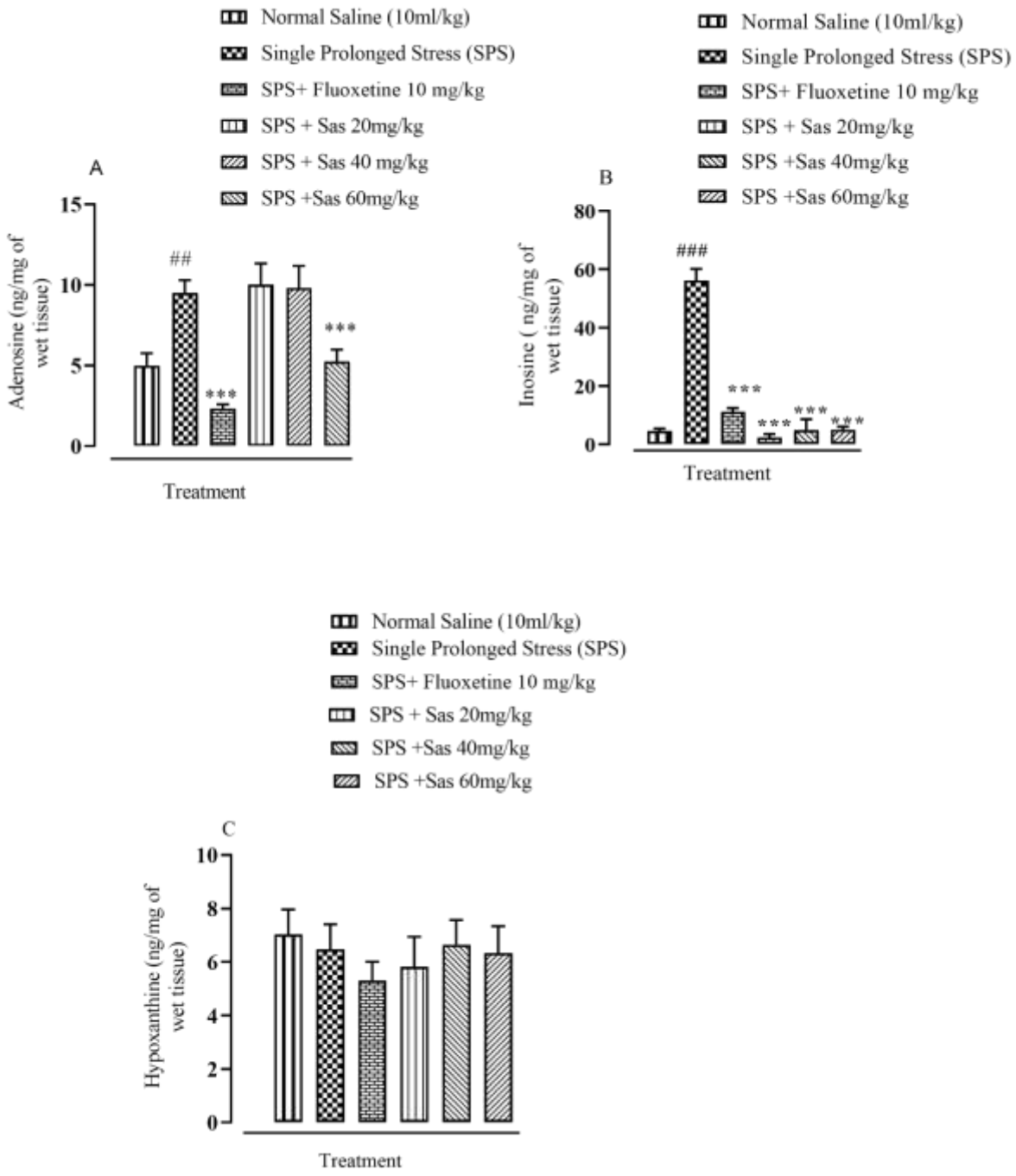

Figure 4. Effect of treatment of Sas or Fluoxetine changes in adenosine, inosine, and hypoxanthine (ng/mg of Wet Tissue) in the frontal cortex after SPS. Difference of significances ###p<0.001 vs Saline group, *p<0.01, **p<0.01, ***p<0.001 vs SPS group.

3.5 Effect of Sas on changes in hippocampal adenosine, inosine, and hypoxanthine levels induced by SPS.

In SPS exposed mice no significant difference was observed in adenosine and hypoxanthine levels in SPS mice in contrast to saline treated groups (Fig 5A, Fig 5C). However, inosine levels were increased significantly in SPS exposed group in comparison with saline treated group which were attenuated by Fluoxetine and Sas at highest dose (Fig 5B).

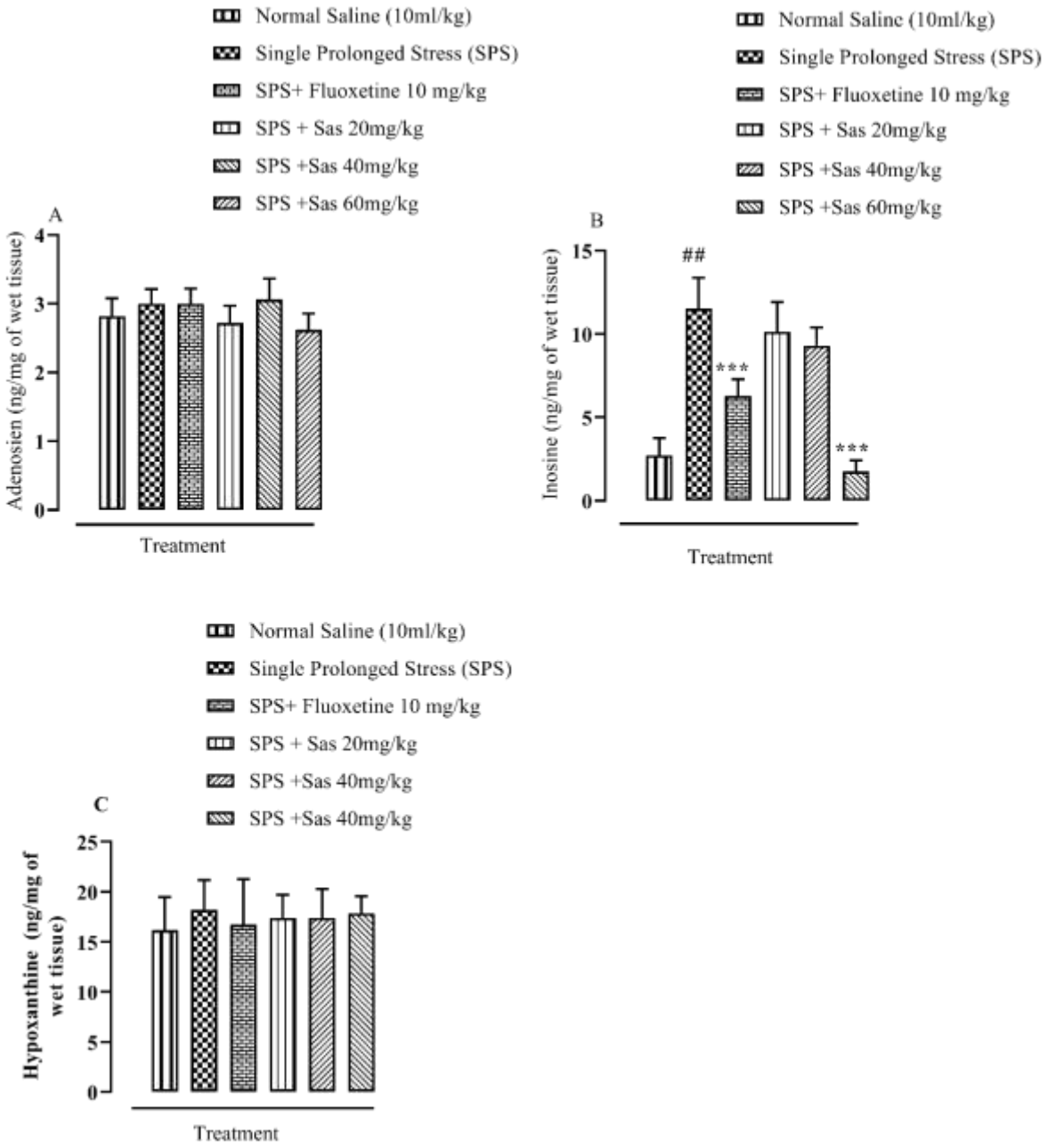

Figure 5. Effect of treatment of Sas or Fluoxetine on changes in adenosine, inosine, and hypoxanthine (ng/mg of Wet Tissue) in the hippocampus after SPS. Difference of significances ###p<0.001 vs Saline group, *p<0.01, **p<0.01, ***p<0.001 vs SPS group

3.6 Effect of Sas on changes in striatal adenosine, inosine, and hypoxanthine levels induced by SPS.

In SPS exposed mice, adenosine was significantly increased in striatum in contrast to saline treated groups which were restored by Fluoxetine and Sas at highest doses (Fig 6A). However, no remarkable difference was observed in inosine and hypoxanthine levels in SPS exposed group in comparison with saline group (Fig 6B, Fig 6C).

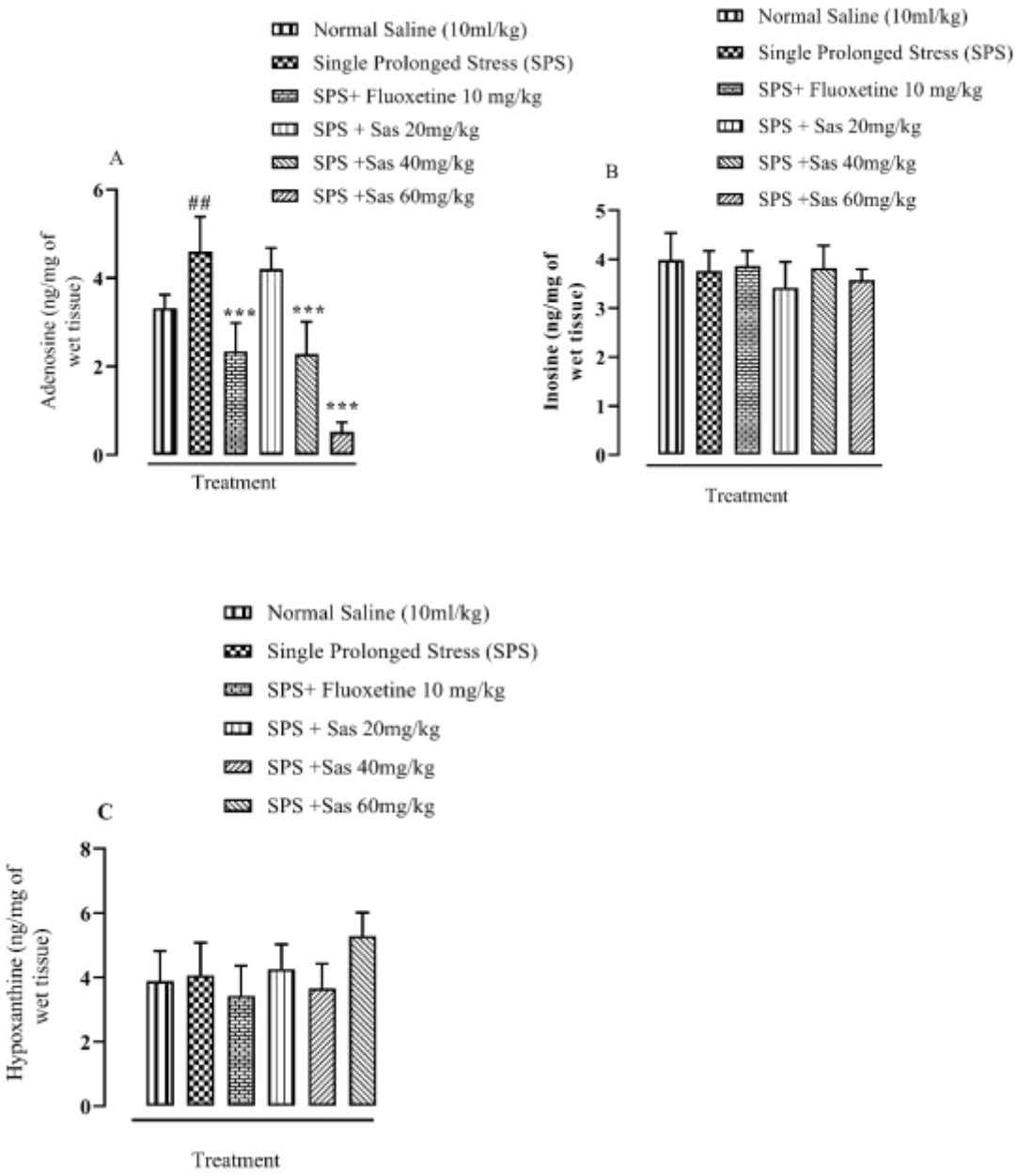

Figure.6 Effect of treatment of Sas or Fluoxetine treatment on changes in adenosine, inosine, and hypoxanthine (ng/mg of Wet Tissue) in the Str after SPS. Difference of significances ###p<0.001 vs Saline group, *p<0.01, **p<0.01, ***p<0.001 vs SPS group.

4 Discussion

The current study was designed to evaluate the effects of Sas on depression and anxiety-like behavior in a mouse model of SPS-induced PTSD. Animal models are crucial for evaluating potential pharmacotherapeutic approaches for PTSD. The SPS model was used in this study to

assess the development of PTSD symptoms. The SPS model was chosen because it generates potential stress responses by means of different mechanisms, that is, immobilization for psychological stress, forced swim for physiological stress, and for pharmacological stress ether was used [49]. The goal of this protocol was to imitate the significant cortisol increase generated by exposure to traumatic experiences [50]. SPS is one of the most effective paradigms for inducing PTSD-related symptoms in rodents, including behavioral despair and anxiety [42].

In the present study, the TST model was used to evaluate the antidepressant activity of Sas in PTSD. The TST is based on the finding that rodent when placed in an inescapable stressful condition, adopt an immobile posture after initial intended escape movements. The stressful situation in the TST includes the hemodynamic stress of being hung in an unpredictable manner by their tail [51]. The depressed mice will give up early and remain immobile for a longer period compared to non-depressed animals [52]. In our findings, immobility time was increased in the TST in mice exposed to SPS, which is an indicator of behavioral despair. These findings are consistent with previous findings [53], however, Sas was able to reduce the immobility time in SPS mice, like fluoxetine (positive control), indicating an antidepressant effect.

Anxiety like behavior was evaluated using MBT. Rodents instinct to bury hostile sources of distress in their territory [54]. This distinctive behavior is normally directed toward detrimental and harmful objects such as unpleasant food, dead conspecifics [55] and small predators like scorpions [56], which are characterized as defensive behaviors that reveal the anxiety state in rodents [57]. In the present study, mice exposed to SPS had buried significantly higher number of marbles than the saline-treated group, indicating anxious behavior. Fluoxetine and Sas significantly lowered marbles burying after SPS exposure, suggesting an anxiolytic effect (Fig 2). In addition, FPO is associated with increased anxiety [46], as it affects the brain gut axis [58]. Increased fecal transit time and FPO are used as the indicators of the modulation of autonomic system in human as well as rodents [46]. However, no significant change was observed among the stressed and non-stressed groups, although the stressed group showed the highest mean FPO among all the groups (Fig3).

Moreover, there was a significant increase in adenosine levels in Frontal cortex in SPS group when compared to saline group which was reversed by fluoxetine and SAS at the highest dose (Fig4 A), however, no significant difference in adenosine levels were observed in hippocampus and Striatum (Fig 5A, Fig 6A). Inosine levels were also raised in the frontal cortex and hippocampus in the SPS

group. Fluoxetine and SAS reduced the inosine levels at all doses in frontal cortex (Fig 4B), and at highest dose in hippocampus (Fig 5B). In contrast, hypoxanthine levels were not altered in any of the brain regions (Fig 5C, Fig 6C, Fig 6D). Hence, the antidepressant and anxiolytic effects of Sas can be correlated with modulation of the adenosergic system.

The association of the adenosergic system with mood disorders has primarily been established, employing adenosine and its analogs to cause depression-like behavioral effects in extensively used animal models of depression [59-61]. Thus, an upsurge in adenosine levels prolonged the immobilization time in rats presented with inescapable shocks and in the FST [62, 63]. Adenosine exerts its actions by acting on the A1 and A2 receptors [17]. The expression of A1 and A2 receptors varies depending on physiological conditions, as it is evident that adenosine mainly acts on A1 receptors under normal physiological conditions due to the high distribution and expression of A1 receptors in the brain, suggesting its role in the maintenance of tone and homeostasis; however, A2ARs are expressed primarily during stress and are involved in fine tuning during some pathologies [35, 64, 65]. This difference in the expression of receptors may be attributed to various factors, such as alteration in neuronal firing pattern, relative position of adenosine discharge, and receptor distribution [17, 66-68]. Increased anhedonia, behavioral despair, and anxiety-like behavior were associated with A2AR overexpression in forebrain neurons in transgenic rats [69]. Hippocampal glutamatergic terminals in mice exhibit increased A2ARs, decreased synaptic plasticity, synaptic protein density, and depressive-like behavior under mild chronic stress [32, 70]. Synaptic and behavioral alterations caused by chronic stress are mitigated by A2AR [70]. The selective A2A receptor antagonist caffeine attenuates depressive behavior in experimental animals, and DMPX augments the antidepressant effect of drugs such as agomelatine and tianeptine [17]. Caffeine has also been reported to attenuate the anxiety like behavior at low doses. Regular exercise exerts an anxiolytic effect by blocking the A2AR receptor [71]. Previous studies have shown that adenosine reduces serotonin release in synapses via the A1 receptor [72] a key neurotransmitter that is involved in the pathophysiology of depression [2, 73], and anxiety [74] whereas the release of acetylcholine and glutamate is facilitated by the activation of A2A receptors whereas the GABAergic release is inhibited [75]. These findings suggest a positive correlation between increased adenosine levels and depression and anxiety-like behavior. Adenosine is converted into inosine by adenosine deaminase, which is further metabolized to hypoxanthine in the presence of purine nucleoside phosphorylase, then to xanthine, and finally to uric acid in the

presence of xanthine oxidase. Inosine is formed inside the cell by adenosine deamination in the presence of a high level of intracellular adenosine [76, 77]. Adenosine and inosine target the same nucleoside transporters [78]. Therefore, by blocking adenosine uptake, inosine can increase adenosine levels in extracellular spaces and exert indirect biological effects. It has been reported that inosine can directly bind to A1, A2A, and A3 receptors. Rodent A3 receptors appear to be more sensitive to the biological effects of inosine than are human A3 receptors [64, 79, 80]. The activation of A3 receptors by inosine has an additional anti-inflammatory effect [76]. However, when the inosine and hypoxanthine levels are abnormally high, they are ultimately metabolized to uric acid, which is responsible for the generation of hydrogen peroxide and potentially deleterious ROS. ROS are responsible for oxidative stress in the brain, which in turn imparts neurodegeneration in the brain [81], ultimately exacerbating mood disorders. Sas modulates the adenosergic pathway by attenuating the adenosine and inosine levels thereby imparting its antidepressant and anxiolytic effects.

Conclusion

In summary, our findings suggest that Sas exerts both antidepressant and anxiolytic effects. Furthermore, Sas modulates the adenosergic pathway, suggesting its possible role in ameliorating depression and anxiety-like behavior. Further studies are required to investigate the exact mechanisms underlying PTSD-induced mood disorders.

Limitations

This study only involved the behavioral aspects of PTSD and neurochemical changes. Deeper insights at the molecular level are required to explore the mechanism of Sas in PTSD.

References

1. Wheaton, M.G., T.-H. Choo, and J.C. Markowitz, *Changes in avoidance and distress related to trauma reminders in PTSD psychotherapy.* Journal of Behavior Therapy and Experimental Psychiatry, 2023. **78**: p. 101805.
2. Yunitri, N., et al., *Global prevalence and associated risk factors of posttraumatic stress disorder during COVID-19 pandemic: A meta-analysis.* International Journal of Nursing Studies, 2022. **126**: p. 104136.
3. Shah, S.F.A., et al., *Trauma exposure and post-traumatic stress disorder among regional journalists in Pakistan.* Journalism, 2022. **23**(2): p. 391-408.
4. Cénat, J.M., et al., *Prevalence of current PTSD symptoms among a sample of Black individuals aged 15 to 40 in Canada: The major role of everyday racial discrimination, racial microaggresions, and internalized racism.* The Canadian Journal of Psychiatry, 2023. **68**(3): p. 178-186.

5. Kakehi, R., et al., *Hypothalamic-pituitary-adrenal axis and renin-angiotensin-aldosterone system in adulthood PTSD and childhood maltreatment history.* Frontiers in Psychiatry, 2023. **13**: p. 967779.
6. Quinones, M.M., et al., *Dysregulation of inflammation, neurobiology, and cognitive function in PTSD: an integrative review.* Cognitive, Affective, & Behavioral Neuroscience, 2020. **20**(3): p. 455-480.
7. Cohen, H. and R. Yehuda, *Gender differences in animal models of posttraumatic stress disorder.* Disease Markers, 2011. **30**(2-3): p. 141-150.
8. Falconer, E., et al., *The neural networks of inhibitory control in posttraumatic stress disorder.* Journal of Psychiatry and Neuroscience, 2008. **33**(5): p. 413-422.
9. Piggott, V.M., et al., *Single-prolonged stress impairs prefrontal cortex control of amygdala and striatum in rats.* Frontiers in Behavioral Neuroscience, 2019. **13**: p. 18.
10. Felmingham, K., et al., *Duration of posttraumatic stress disorder predicts hippocampal grey matter loss.* Neuroreport, 2009. **20**(16): p. 1402-1406.
11. Wilson, C.B., et al., *Predator exposure/psychosocial stress animal model of post-traumatic stress disorder modulates neurotransmitters in the rat hippocampus and prefrontal cortex.* PloS One, 2014. **9**(2): p. e89104.
12. Hayes, J.P., et al., *Reduced hippocampal and amygdala activity predicts memory distortions for trauma reminders in combat-related PTSD.* Journal of Psychiatric Research, 2011. **45**(5): p. 660-669.
13. Ma, W.-X., et al., *Adenosine and P1 receptors: Key targets in the regulation of sleep, torpor, and hibernation.* Frontiers in Pharmacology, 2023. **14**: p. 614.
14. Latini, S. and F. Pedata, *Adenosine in the central nervous system: release mechanisms and extracellular concentrations.* Journal of Neurochemistry, 2001. **79**(3): p. 463-484.
15. Gomes, C.V., et al., *Adenosine receptors and brain diseases: neuroprotection and neurodegeneration.* Biochimica et Biophysica Acta (BBA)-Biomembranes, 2011. **1808**(5): p. 1380-1399.
16. Elmenhorst, E.-M., et al., *Cognitive impairments by alcohol and sleep deprivation indicate trait characteristics and a potential role for adenosine A1 receptors.* Proceedings of the National Academy of Sciences, 2018. **115**(31): p. 8009-8014.
17. van Calker, D., et al., *The role of adenosine receptors in mood and anxiety disorders.* Journal of Neurochemistry, 2019. **151**(1): p. 11-27.
18. Pacella, M.L., B. Hruska, and D.L. Delahanty, *The physical health consequences of PTSD and PTSD symptoms: a meta-analytic review.* Journal of Anxiety Disorders, 2013. **27**(1): p. 33-46.
19. Watkins, L.E., K.R. Sprang, and B.O. Rothbaum, *Treating PTSD: A review of evidence-based psychotherapy interventions.* Frontiers in Behavioral Neuroscience, 2018. **12**: p. 258.
20. Draper, B. and K. Berman, *Tolerability of selective serotonin reuptake inhibitors: issues relevant to the elderly.* Drugs & aging, 2008. **25**: p. 501-519.
21. Wahbeh, H., et al., *Mechanistic pathways of mindfulness meditation in combat veterans with posttraumatic stress disorder.* Journal of Clinical Psychology, 2016. **72**(4): p. 365-383.
22. Akiki, T.J. and C.G. Abdallah, *Are there effective psychopharmacologic treatments for PTSD?* The Journal of Clinical Psychiatry, 2019. **80**(3): p. 1309.
23. Huang, Z.-L., Y. Urade, and O. Hayaishi, *The role of adenosine in the regulation of sleep.* Current Topics in Medicinal Chemistry, 2011. **11**(8): p. 1047-1057.
24. Canas, P.M., et al., *Neuronal adenosine A2A receptors are critical mediators of neurodegeneration triggered by convulsions.* Eneuro, 2018. **5**(6).

25. Pan, W.J., S. Osmanović, and S.A. Shefner, *Characterization of the adenosine A1 receptor-activated potassium current in rat locus ceruleus neurons.* Journal of Pharmacology and Experimental Therapeutics, 1995. **273**(1): p. 537-544.
26. Vincenzi, F., et al., *Positive allosteric modulation of A1 adenosine receptors as a novel and promising therapeutic strategy for anxiety.* Neuropharmacology, 2016. **111**: p. 283-292.
27. Vincenzi, F., P.A. Borea, and K. Varani, *Anxiolytic properties of A1 adenosine receptor PAMs.* Oncotarget, 2017. **8**(5): p. 7216.
28. Stollenwerk, T.M., S. Pollock, and C.J. Hillard, *Contribution of the adenosine 2A receptor to behavioral effects of tetrahydrocannabinol, cannabidiol and PECS-101.* Molecules, 2021. **26**(17): p. 5354.
29. Cao, C., et al., *Untargeted Metabolomic Analysis Reveals the Metabolic Disturbances and Exacerbation of Oxidative Stress in the Cerebral Cortex of a BTBR Mouse Model of Autism.* Journal of Molecular Neuroscience, 2023. **73**(1): p. 15-27.
30. Jarrar, Q., et al., *Anxiolytic Effect of Ethanolic Extract of Medjool Dates of Phoenix Dactylifera in Mice.* Jordan Journal of Pharmaceutical Sciences, 2023. **16**(1): p. 46-60.
31. Żmudzka, E., et al., *Novel Multimodal Salicylamide Derivative with Antidepressant-like, Anxiolytic-like, Antipsychotic-like, and Anti-Amnesic Activity in Mice.* Pharmaceuticals, 2023. **16**(2): p. 175.
32. Pasquini, S., et al., *Adenosine receptors in neuropsychiatric disorders: fine regulators of neurotransmission and potential therapeutic targets.* International Journal of Molecular Sciences, 2022. **23**(3): p. 1219.
33. Boison, D., *Adenosine as a neuromodulator in neurological diseases.* Current Opinion in Pharmacology, 2008. **8**(1): p. 2-7.
34. Oliveira, S., et al., *Impact of genetic variations in ADORA2A gene on depression and symptoms: a cross-sectional population-based study.* Purinergic Signalling, 2019. **15**: p. 37-44.
35. Cunha, R., *Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors.* Neurochemistry International, 2001. **38**(2): p. 107-125.
36. Moreira-de-Sá, A., et al., *Adenosine A2A receptors as biomarkers of brain diseases.* Frontiers in Neuroscience, 2021. **15**: p. 702581.
37. Cunha, G., et al., *Increased density and synapto-protective effect of adenosine A2A receptors upon sub-chronic restraint stress.* Neuroscience, 2006. **141**(4): p. 1775-1781.
38. Hu, Y., et al., *A new approach to the pharmacological regulation of memory: Sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models.* Brain Research, 2005. **1060**(1-2): p. 26-39.
39. Ren, L.-X., et al., *Antidepressant-Like Effects of Sarsasapogenin from Anemarrhena asphodeloides B UNGE (Liliaceae).* Biological and Pharmaceutical Bulletin, 2006. **29**(11): p. 2304-2306.
40. Kong, L., et al., *Sarsasapogenin ameliorates diabetes-associated memory impairment and neuroinflammation through down-regulation of PAR-1 receptor.* Phytotherapy Research, 2021. **35**(6): p. 3167-3180.
41. Zhang, M.-Y., et al., *Sarsasapogenin suppresses Aβ overproduction induced by high glucose in HT-22 cells.* Naunyn-Schmiedeberg's Archives of Pharmacology, 2018. **391**: p. 159-168.
42. Souza, R.R., L.J. Noble, and C.K. McIntyre, *Using the single prolonged stress model to examine the pathophysiology of PTSD.* Frontiers in pharmacology, 2017. **8**: p. 615.
43. Tian, X., et al., *9.4 T static magnetic field ameliorates imatinib mesylate-induced toxicity and depression in mice.* European Journal of Nuclear Medicine and Molecular Imaging, 2023. **50**(2): p. 314-327.
44. Angoa-Pérez, M., et al., *Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice.* Journal of Visualized Experiments: JoVE, 2013(82).

45. Liu, C., et al., *Microglial transglutaminase 2 deficiency causes impaired synaptic remodelling and cognitive deficits in mice.* Cell Proliferation, 2023: p. e13439.
46. Valdivieso, D.A., et al., *Effects of pregabalin on neurobehavior in an adult male rat model of PTSD.* PloS One, 2018. **13**(12): p. e0209494.
47. Arif, M., et al., *6-Methoxyflavone and donepezil behavioral plus neurochemical correlates in reversing chronic ethanol and withdrawal induced cognitive impairment.* Drug Design, Development and Therapy, 2022. **16**: p. 1573.
48. Ur Rehman, N., et al., *Effect of 4-Fluoro-N-(4-Sulfamoylbenzyl) benzene sulfonamide on acquisition and expression of nicotine-induced behavioral sensitization and striatal adenosine levels.* Drug Design, Development and Therapy, 2020: p. 3777-3786.
49. Lisieski, M.J., et al., *Single-prolonged stress: a review of two decades of progress in a rodent model of post-traumatic stress disorder.* Frontiers in Psychiatry, 2018. **9**: p. 196.
50. Armario, A., R.M. Escorihuela, and R. Nadal, *Long-term neuroendocrine and behavioural effects of a single exposure to stress in adult animals.* Neuroscience & Biobehavioral Reviews, 2008. **32**(6): p. 1121-1135.
51. Cryan, J.F., C. Mombereau, and A. Vassout, *The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice.* Neuroscience & Biobehavioral Reviews, 2005. **29**(4-5): p. 571-625.
52. Liao, J.-C., et al., *Antidepressant-like activity of turmerone in behavioral despair tests in mice.* BMC Complementary and Alternative Medicine, 2013. **13**: p. 1-8.
53. El-Elimat, T., et al., *Silymarin prevents memory impairments, anxiety, and depressive-like symptoms in a rat model of post-traumatic stress disorder.* Planta Medica, 2019. **85**(01): p. 32-40.
54. Archer, T., et al., *Marble burying and spontaneous motor activity in mice: interactions over days and the effect of diazepam.* Scandinavian journal of Psychology, 1987. **28**(3): p. 242-249.
55. Treit, D., *A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics.* Pharmacology Biochemistry and Behavior, 1990. **36**(1): p. 203-205.
56. Londei, T., A.M. Valentini, and V.G. Leone, *Investigative burying by laboratory mice may involve non-functional, compulsive, behaviour.* Behavioural Brain Research, 1998. **94**(2): p. 249-254.
57. Nicolas, L.B., Y. Kolb, and E.P. Prinssen, *A combined marble burying–locomotor activity test in mice: A practical screening test with sensitivity to different classes of anxiolytics and antidepressants.* European Journal of Pharmacology, 2006. **547**(1-3): p. 106-115.
58. Savignac, H., et al., *B ifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice.* Neurogastroenterology & Motility, 2014. **26**(11): p. 1615-1627.
59. Woodson, J.C., T.R. Minor, and R.S. Job, *by Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) Mimics the Effect of Inescapable Shock on Escape Learning in Rats.* 1998.
60. Hunter, A.M., B.W. Balleine, and T.R. Minor, *Helplessness and escape performance: glutamate-adenosine interactions in the frontal cortex.* Behavioral Neuroscience, 2003. **117**(1): p. 123.
61. Cunha, R.A., et al., *Potential therapeutic interest of adenosine A2A receptors in psychiatric disorders.* Current Pharmaceutical Design, 2008. **14**(15): p. 1512-1524.
62. Yamada, K., M. Kobayashi, and T. Kanda, *Involvement of adenosine A2A receptors in depression and anxiety.* International Review of Neurobiology, 2014. **119**: p. 373-393.
63. Kulkarni, S. and A.K. Mehta, *Purine nucleoside—mediated immobility in mice: reversal by antidepressants.* Psychopharmacology, 1985. **85**: p. 460-463.
64. Fredholm, B.B., et al., *Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells.* Biochemical Pharmacology, 2001. **61**(4): p. 443-448.
65. Shen, H.-Y. and J.-F. Chen, *Adenosine A2A receptors in psychopharmacology: modulators of behavior, mood and cognition.* Current Neuropharmacology, 2009. **7**(3): p. 195-206.

66. James, S. and P.J. Richardson, *The subcellular distribution of [3H]-CGS 21680 binding sites in the rat striatum: copurification with cholinergic nerve terminals.* Neurochemistry international, 1993. **23**(2): p. 115-122.
67. Cunha, R.A., et al., *Evidence for high-affinity binding sites for the adenosine A 2A receptor agonist [3 H] CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal A 2A receptors.* Naunyn-Schmiedeberg's Archives of Pharmacology, 1996. **353**: p. 261-271.
68. Sebastiao, A. and J. Ribeiro, *Adenosine A2 receptor-mediated excitatory actions on the nervous system.* Progress in Neurobiology, 1996. **48**(3): p. 167-189.
69. Coelho, J.E., et al., *Overexpression of adenosine A2A receptors in rats: effects on depression, locomotion, and anxiety.* Frontiers in Psychiatry, 2014. **5**: p. 67.
70. Kaster, M.P., et al., *Caffeine acts through neuronal adenosine A2A receptors to prevent mood and memory dysfunction triggered by chronic stress.* Proceedings of the National Academy of Sciences, 2015. **112**(25): p. 7833-7838.
71. Leem, Y.-H., et al., *Exercise exerts an anxiolytic effect against repeated restraint stress through 5-HT2A-mediated suppression of the adenosine A2A receptor in the basolateral amygdala.* Psychoneuroendocrinology, 2019. **108**: p. 182-189.
72. Okada, M., et al., *Adenosine receptor subtypes modulate two major functional pathways for hippocampal serotonin release.* Journal of Neuroscience, 2001. **21**(2): p. 628-640.
73. Okada, M., et al., *Differential effects of adenosine receptor subtypes on release and reuptake of hippocampal serotonin.* European Journal of Neuroscience, 1999. **11**(1): p. 1-9.
74. Zangrossi Jr, H. and F.G. Graeff, *Serotonin in anxiety and panic: contributions of the elevated T-maze.* Neuroscience & Biobehavioral Reviews, 2014. **46**: p. 397-406.
75. Sperlágh, B. and E. Sylvester Vizi, *The role of extracellular adenosine in chemical neurotransmission in the hippocampus and Basal Ganglia: pharmacological and clinical aspects.* Current Topics in Medicinal Chemistry, 2011. **11**(8): p. 1034-1046.
76. Haskó, G., M.V. Sitkovsky, and C. Szabo, *Immunomodulatory and neuroprotective effects of inosine.* Trends in Pharmacological Sciences, 2004. **25**(3): p. 152-157.
77. Boison, D. and G.G. Yegutkin, *Adenosine metabolism: emerging concepts for cancer therapy.* Cancer cell, 2019. **36**(6): p. 582-596.
78. Kaur, T., et al., *Nucleoside transporters and immunosuppressive adenosine signaling in the tumor microenvironment: Potential therapeutic opportunities.* Pharmacology & Therapeutics, 2022: p. 108300.
79. Jin, X., et al., *Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation.* The Journal of Clinical Investigation, 1997. **100**(11): p. 2849-2857.
80. Tilley, S.L., et al., *Adenosine and inosine increase cutaneous vasopermeability by activating A 3 receptors on mast cells.* The Journal of clinical investigation, 2000. **105**(3): p. 361-367.
81. Frenguelli, B.G. and N. Dale, *Purines: from diagnostic biomarkers to therapeutic agents in brain injury.* Neuroscience Bulletin, 2020. **36**: p. 1315-1326.

Authors Contribution Statement

First Author

Hurmat Malik[1] 0009-0007-4722-5599

PhD Research Scholar

[1]Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan

Second Author

Dr. Gowhar Ali[2]

Post Doc and PhD Pharmacology

[2]Department of Pharmacy University of Peshawar, KP Pakistan, Pakistan

Third Author

Muhammad Usman[1]

PhD Research Scholar

[1]Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan

Fourth Author

Dr. Mehreen Arif [1]

PhD Pharmacology

[1]Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan

Fifth Author

Zainab Ahmed [1]

PhD Research Scholar

[1]Department of Pharmacy, COMSATS University Islamabad, Pakistan

Sixth Author

Dr. Muhammad Sona Khan[1]

PhD Pharmacology

[1]Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan

Seventh Author

Corresponding author*

Dr. Khalid Rauf 0000-0003-3967-9623

PhD Pharmacology

[1]Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan

Eighth Author

Prof Robert D. E. Sewell[3]

PhD Pharmacology

[3]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, UK

Conflict of interest:

The authors declare no competing interest.

Funding:

Any agency did not fund the current research work.

Submission declaration:

The work described above has not been published formerly and it is not under consideration for publication somewhere else. All authors approved the publication and if accepted in this journal, will not be published elsewhere.