## Abstract

Post-traumatic stress disorder (PTSD) is a neuropsychiatric disorder, which develops in reaction to an actual or perceived traumatic event. According to DSM-5, the characteristic symptoms of PTSD include hyperarousal, fear response, and recurrence of aversive thoughts. The pathophysiology of PTSD includes abnormalities in hypothalamic-pituitary-adrenal (HPA)-axis function and monoaminergic system. Pharmacotherapy of PTSD involves selective serotonin reuptake inhibitors (SSRIs) such as sertraline and paroxetine based on the monoaminergic theory. However, these drugs are useful only for 30 percent of PTSD patients and are effective only against limited symptoms of PTSD. Therefore, it is essential to evaluate novel targets for the pharmacotherapy of PTSD. Clinical studies have shown the role of the orexinergic system in PTSD. However, there is a paucity of evidence on the functioning of the orexinergic system in animal models of PTSD. Therefore, evaluating the role of the orexinergic system in an animal model may provide preclinical evidence for the treatment of PTSD. The stress re-stress (SRS) model of PTSD using the foot shock (FS) as a re-stress was used for the pharmacological evaluation of orexin-A in the experimental model of PTSD. Male Wistar rats were subjected to SRS by restraining them for 2 h followed by FS (2mA for 10 sec) and halothane exposure on day-2 (D-2). Then the rats were weekly exposed to FS as a re-stress cue up to D-32. Behavioural assessments such as freezing behaviour, anxiety-like behaviour, and cognitive behaviour were performed on D-8 and D-32. Suvorexant (10, 20, and 30 mg/kg *po*) and paroxetine (10 mg/kg *po*) was administered from D-8 to D-32. Plasma and cerebrospinal fluid (CSF) were collected for corticosterone and orexin-A measurement. The analysis of serotonin and corticotropin-releasing factor receptor-1 (CRF-R1) was performed in the amygdalar tissue. SRS-induced PTSD-like symptoms such as fear response, anxiety-like behaviour, and hypocorticosteronism were attenuated

by suvorexant and paroxetine. Interestingly, SRS exposed rats showed activation of orexin-A and serotonergic systems which were also attenuated by suvorexant. Additionally, suvorexant ameliorated the extrahypothalamic induced upregulation of CRH-R1 in SRS-exposed rats. The current findings indicated that that unlike paroxetine, suvorexant counteracted the hyperactivity of the orexinergic system in the SRS model by antagonizing orexinergic receptor non-selectively. Further, suvorexant improved the HPA-axis function and CRH system by blocking the malfunctioning of orexin-A. Suvorexant also alleviated the serotonergic hyperactivity in the SRS model and attenuated PTSD-like symptoms. Moreover, the suvorexant-induced anti-PTSD-like effect was comparable to paroxetine. Suvorexant but not paroxetine also attenuated SRS-induced alteration in locomotion and also produced a sedative effect on unstressed rats.

Cognitive inflexibility is one of the major clinical symptoms of PTSD. The cholinergic system mainly regulates cognitive flexibility, and there were limited reports on the effect of traumatic episodes on cognitive flexibility. Therefore, animal models developing cognitive inflexibility would provide new insight into the pathophysiology and pharmacotherapy of PTSD. SRS model was used (similar to the objective-1) to evaluate the cholinergic system's involvement and for the assessment of cognitive inflexibility. Then the rats were exposed every week to FS as a re-stress cue up to D-32. Donepezil (3 mg/kg; *po*) and sertraline (10 mg/kg; *po*) dosing was started from D-8 and continued up to D-32. SRS exposure caused cognitive inflexibility by producing intra-dimension (ID) deficits and extra-dimension (ED) set-shifting, which was significantly attenuated by donepezil. However, sertraline mitigated only ID shift in SRS subjected rats. Both donepezil and sertraline attenuated SRS-induced PTSD-like symptoms such as fear response, anxiety-like behaviour, and cognitive deficits. Donepezil did not modulate the SRS-induced HPA-axis dysfunction and activation of the serotonergic and noradrenergic

systems. Interestingly, exposure to SRS caused a decrease in acetylcholine level and increased acetylcholine esterase activity in the prefrontal cortex (PFC) and hippocampus (HIP) which was only mitigated by donepezil. Donepezil significantly attenuated SRS-induced down-regulation of choline-acetyl transferase and α-7nicotinic acetylcholine receptor expressions in PFC and HIP. In conclusion, cognitive inflexibility is developed in the SRS model, along with other PTSD-like symptoms that were attenuated by donepezil.

Further, growing evidence suggested that mitochondrial dysfunction is another pathological cause of PTSD. The mitochondrial dynamic plays an essential role in regulating energy balance, mitochondrial biogenesis, and homeostasis during a stressful situation. However, there is paucity on the role of mitochondrial dynamics in the experimental model of PTSD. The study's objective is to determine the effect of orexin antagonist on mitochondrial dynamics in rats exhibiting PTSD-like symptoms. Exposure of SRS caused activation of mammalian target of rapamycin complex 1 (mTORC-1) pathway and associated stimulation of mitochondrial fission process (MTFP-1). MTFP-1 protein is responsible for the modulation of mitochondrial dynamics by enhancing mitochondrial fission. Therefore, mitochondrial dynamics parameters such as protein and mRNA expression of mitochondrial fission marker dynamin-related protein (Drp-1), mitochondrial fission protein-1 (Fis-1), and fusion marker optic atrophy-1 (OPA-1) and mitofusin-2 (Mfn-2) were analysed. Immunohistochemistry of mitochondrial fission (Drp-1) and fusion (Mfn-2) was performed to confirm mito-dynamics markers' localization in the amygdala. Further, the alteration in mitochondrial dynamics can deregulate mitophagy, which was analysed through the TEM method. The salient finding is a disparity in mitochondrial dynamics as observed by enhanced fission regulating protein (Drp-1 and Fis-1) and reduced fusion regulating protein (Opa-1 and Mfn-2)

following exposure to SRS. The alteration in mitochondrial dynamics is further conformed by immunofluorescence assay, as observed by an increase in the intensity of Drp-1 and a decrease in the intensity of Mfn-2 co-localisation in the AMY. The modulation in mitochondrial dynamics is mediated by the activation mTORC-1 pathway and translation of MTFP-1, which promotes mitochondrial fission by phosphorylating Drp-1. Exposure to SRS caused fewer mitophagy vacuoles with more swollen and damaged mitochondria observed during TEM analysis. Treatment with suvorexant and rapamycin significantly improved mitochondrial dynamics and mitochondrial bioenergetics in SRS-exposed rats. Further, suvorexant and rapamycin significantly mitigated the activation of mTORC-1 and MTFP-1. Furthermore, combination-induced attenuation is more significant than individual treatment. Sertraline did not cause any effect on SRS-induced activation of mTORC-1 and MTFP-1. Moreover, suvorexant promotes the elimination of non-functional mitochondria through mTOR dependent pathway and promotes autophagosome formation for final degradation.

Together the findings suggested that suvorexant showed anti-PTSD-like effect, which was comparable to paroxetine and sertraline. Suvorexant attenuated SRS-induced cognitive inflexibility and cholinergic hypoactivity. Treatment with suvorexant alleviated the PTSD-like phenotypes as well as mitochondrial dysfunction through mTORC-1 dependent pathway. Therefore, suvorexant may be an ideal treatment candidate for PTSD.