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The Effect of NKCC1 Inhibitor Azosemide on Emotional Behaviors and Hippocampal GABA Levels in a Rat Model of Post-traumatic Stress Disorder

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ABSTRACT

Post-traumatic stress disorder (PTSD) is an anxiety disorder that can occur immediately or years after exposure to a traumatic event. Despite extensive research, the etiology of PTSD is largely unknown, but it is thought that impaired GABAergic transmission may play a role in the disease process. Using a single prolonged stress (SPS) procedure, we aimed to determine the effect of azosemide, a sodium-potassium-chloride cotransporter (NKCC1) inhibitor, on anxiety and memory-related behaviors and hippocampal GABA level in rats. Behavioral tests were performed by open field test and passive avoidance test, while hippocampal GABA levels were determined by ELISA. We found that azosemide treatment partially improved emotional behavior and significantly improved memory performance in rats with PTSD, without affecting the decline in hippocampal GABA levels induced by a SPS exposure. These findings suggest that azosemide may offer partial therapeutic benefits for symptoms of PTSD, particularly cognitive deficits. However, they underscore the necessity for multimodal approaches to address the various neurobiological underpinnings of the disorder.

Keywords: Post-traumatic stress disorder, Azosemide, Hippocampus, Rat.

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Introduction

Posttraumatic stress disorder (PTSD) is a psychological condition that can develop following exposure to a traumatic event, such as war, natural disasters, sexual assault or serious accidents [1]. PTSD is characterized by a constellation of symptoms, including intrusive memories, hyperarousal, avoidance behaviors, and negative changes in cognition and mood [2]. These symptoms significantly impair individuals' daily functioning and overall quality of life. PTSD has been recognized as a distinct psychiatric disorder since its inclusion in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) in 1980 [3]. The estimated prevalence of PTSD in the general population ranges from 6 to 8 percent; however, specific populations, such as military veterans and first responders, demonstrate significantly higher rates [4]. For instance, it is estimated that up to 30 percent of military veterans may experience PTSD because of combat-related trauma. In recent years, the significance of PTSD has garnered increased attention, particularly following the COVID-19 pandemic, which has highlighted its effects on healthcare professionals and individuals who have suffered loss [5].

The pathophysiology of PTSD is complex and multifactorial, involving dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, alterations in neurotransmitter systems, and both structural and functional changes in key brain regions, including the amygdala, hippocampus, and prefrontal cortex [6], [7]. These neurobiological changes are believed to underlie the hallmark symptoms of PTSD, including increased reactivity to trauma-related cues and a diminished ability to regulate emotions and manage stress. Recent advancements in neuroimaging and molecular genetics underscore the significant role of both genetic and environmental factors in the development of PTSD. Contemporary treatment approaches generally involve a combination of pharmacotherapy-primarily selective serotonin reuptake inhibitors (SSRIs) and GABAergic medications-along with trauma-focused cognitive behavioral therapy, which is the primary modality of psychotherapy [3]. Recently, emerging modalities, including psychedelics, ketamine, and neuromodulation techniques, have been investigated as potential interventions for treatment-resistant PTSD [8]. Despite the availability of various treatments, many individuals diagnosed with PTSD do not achieve complete recovery. This highlights the necessity for ongoing research into novel therapeutic approaches.

Azosemide is a loop diuretic primarily used to treat conditions characterized by fluid overload, such as heart failure, renal failure, and hypertension [9]. Azosemide operates by inhibiting the sodium-potassium-chloride cotransporter (NKCC1) in the ascending thick limb of the loop of Henle, resulting in increased excretion of sodium, chloride, and water [10]. This inhibition leads to an increased excretion of sodium, chloride, and water. The diuretic effect of azosemide decreases plasma volume and alleviates symptoms associated with fluid retention. Azosemide demonstrates a powerful diuretic effect and

has a longer duration of action compared to furosemide, another loop diuretic. This property allows azosemide to be an effective tool in the clinical treatment of edema and hypertension. However, azosemide has also garnered attention for its ability to restore neurotransmission balance, presenting a novel therapeutic strategy [11], [12]. Specifically, the capacity of azosemide to inhibit NKCC1 within the central nervous system may have broader implications for its application in psychiatric disorders. NKCC1 plays a crucial role in regulating the intracellular chloride concentration ([Cl-]i) within neurons, functioning as a cation-chloride cotransporter that facilitates the accumulation of Cl- ions in the neuronal cytosol [13]. Consequently, NKCC1 is integral to the maintenance of the chloride gradient in neurons, which is essential for the proper functioning of gamma-aminobutyric acid (GABA) receptors. The dysregulation of this gradient may play a significant role in the pathophysiology of PTSD, particularly concerning altered inhibitory neurotransmission and increased neuronal excitability [14]. Azosemide may facilitate the restoration of normal chloride homeostasis through the inhibition of NKCC1, which may subsequently alleviate symptoms of hyperarousal and anxiety associated with PTSD by enhancing GABAergic inhibition. Considering this information, we conducted an investigation into the effects of azosemide treatment on emotional behavior and hippocampal GABA levels in a rat model of PTSD to elucidate the potential preventive role of azosemide in alleviatiating symptoms associated with PTSD.

Material and Method

Animals and Treatments

Eighteen male Wistar Albino rats, each weighing between 200-250 grams, were utilized in this study. The experimental subjects were divided into three distinct groups:

1) Control group; it was not exposed to any stressors,
2) Post-travmatic stress disorder (PTSD) group; it consisted of subjects that were subjected to a SPS exposure,
3) PTSD + Azosemide (PTSD + AZO) group; it consisted of animals that were administered azosemide at a dosage of 5 mg/kg i.p. after trauma exposure.

To create an animal model of PTSD, rats underwent a SPS exposure protocol (Figure 1). Among the various animal models of PTSD that have been used to imitate the basic symptoms, such as anxiety and fear-related behaviors, the SPS protocol has consistently demonstrated good face validity for the PTSD condition in humans. In this model, anxiety-related behavioral disorders are used as an indicator of the severity of the PTSD-like phenotype, and this provides us with a reliable measure to assess the possible contribution of posttraumatic azosemide treatment to the development of PTSD. Initially, the animals were confined in an apparatus that immobilized them for two hours. Subsequently, they were placed in a tank (124 cm in diameter and 32 cm in depth) filled with water at 22°C, where they experienced 20 minutes of forced swimming. Following this, they were placed in an isolated apparatus where they were exposed to ether until unconsciousness occurred [15].

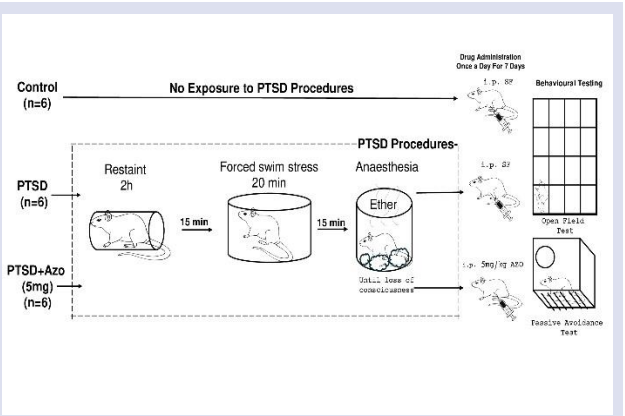

Figure 1. Experimental protocol.

The animals except for the control group were subjected to 2 hours of restraint, 20 minutes of forced swimming stress and ether until unconsciousness, respectively. AZO: Azosemide, PTSD: Post-travmatic stress disorder, PTSD + AZO: Post-Traumatic Stress Disorder + Azosemide.

Previous studies have shown that NKCC inhibitors, including azosemide, penetrate the brain by crossing the blood-brain barrier and act by binding to these receptors [12], [16]. The rats in the PTSD + AZO group were administered azosemide at a dose of 5 mg/kg i.p. for 7 days following exposure to a traumatic event. On the 8th day, behavioral assessments, including open field and passive avoidance tests, were conducted on all subjects. After the 9th day, all animals were euthanasied by ethical guidelines, and hippocampal tissue samples were collected under sterile surgical conditions for the subsequent measurement of GABA levels. All procedures involving the animals were conducted with the approval of the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (Decision No: 65202830-050.04.04-72).

Open Field Test

This experiment was conducted to evaluate the emotional behavior and locomotor activity of animals within a platform measuring 100 cm×100 cm×30 cm, which was subdivided into 16 equal squares. Individual rats were placed in the center of the platform and permitted to move freely for 5 min. During this period, the activities of the animals were recorded via video, and subsequent analyses were performed on the number of frames traversed, as well as instances of rearing and grooming behavior [17].

Passive Avoidance Test

The passive avoidance test is a procedure designed to evaluate fear-related memory in rodents. This test utilizes an apparatus comprising two chambers: one illuminated and the other darkened, which are separated by an

automatically closing door. The floors of both chambers are constructed from stainless steel bars. In the initial phase of the two-day experiment, the rats were situated in a brightly illuminated room, and the door was opened after 10 seconds. Considering that rodents exhibit an inherent preference for darker environments and tend to avoid brightly lit areas, the subjects were observed to progressively move towards the dark chamber of the apparatus. Upon their entry into the dark chamber, the door was subsequently closed, and the animal was administered a single low-intensity foot shock of 0.5 mA for 5 seconds. On the subsequent day, during the retention phase, the rats underwent the same procedure following a 24-hour interval; however, no shock was administered when the animals entered the dark chamber. The transition time of the rats from the light chamber to the dark chamber was recorded on both day one and day two. Learning performance was assessed by measuring the increase in the time delay during the retention trial in comparison to the acquisition trial [18].

Measurement of GABA Levels in the Hippocampus

In the assessment of the impact of azosemide treatment on hippocampal GABA levels, GABA concentrations in hippocampal tissue samples from the subjects were quantified using the enzyme-linked immunosorbent assay (ELISA) method [19]. The hippocampal tissue samples were homogenized in sterile phosphate-buffered saline (PBS) utilizing a mechanical homogenizer (SpeedMill PLUS; Analytik Jena). Subsequently, the homogenates were centrifuged at 4000 rpm for 10 min at a temperature of 4°C. Following the centrifugation, the supernatant was collected for further analysis, and the Bradford protein assay was employed to determine protein concentration. The ELISA protocol was conducted according to the manufacturer's instructions.

Statistical Analysis

GraphPad Prism (version 8.0) software was utilized to analyze the data collected during the study. The data are presented as mean ± standard error of the mean (SEM) and were subjected to a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey test. Results with a p-value of less than 0.05 were deemed statistically significant.

Results and Discussion

Effect of Azosemide on Number of Line Crossed, Grooming and Rearing in the Open Field Test

The effect of azosemide treatment on the number of line crossed, rearing and grooming behavior during the open field test in rats subjected to a SPS is illustrated in Figure 2. It was observed that the number of line crossed by the rats with PTSD was significantly lower than that of the control group ($p < 0.05$). Furthermore, the number of rearing behaviors was significantly reduced in both the PTSD and PTSD + AZO groups when compared to the control group ($p < 0.05$). In addition, the frequency of grooming behaviors was statistically significantly lower in the PTSD group when compared to the control group ($p < 0.05$).

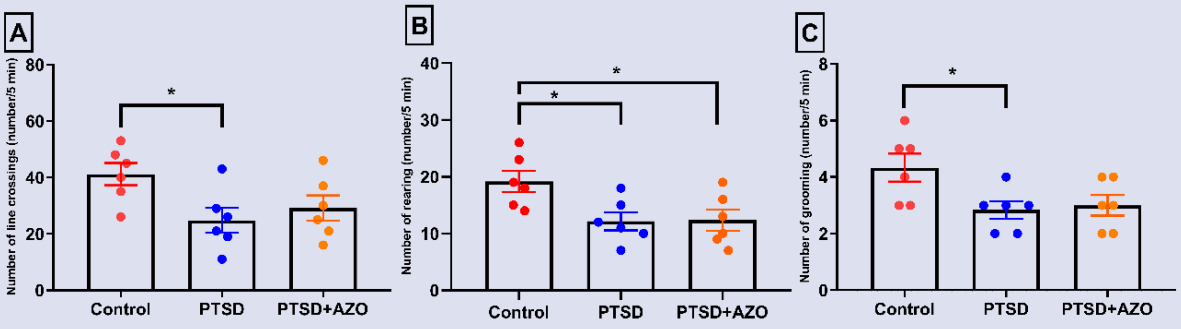

Figure 2. The effect of azosemide treatment on the frequency of line crossed, rearing, and grooming behaviors in the open field test within the PTSD rat model. Data are presented as mean ± standard error of the mean (*$p < 0.05$, compared to the control group; AZO: Azosemide, PTSD: Post-Traumatic Stress Disorder, PTSD + AZO: Post-Traumatic Stress Disorder + Azosemide).

Effect of Azosemide on Emotional Memory Performance in Passive Avoidance Test

The effect of azosemide treatment on emotional memory in rats subjected to SPS was evaluated using the passive avoidance test. During the acquisition phase on the initial day, no statistically significant differences were observed between the groups regarding the time taken to transition from the bright area to the dark area. On the other hand, after 24 hours, during the retention phase on the second day, a significant reduction in the escape time

from the bright area to the dark area was observed in the rats belonging to the PTSD group when compared to those in both the control group and the PTSD + AZO group (Figure 3).

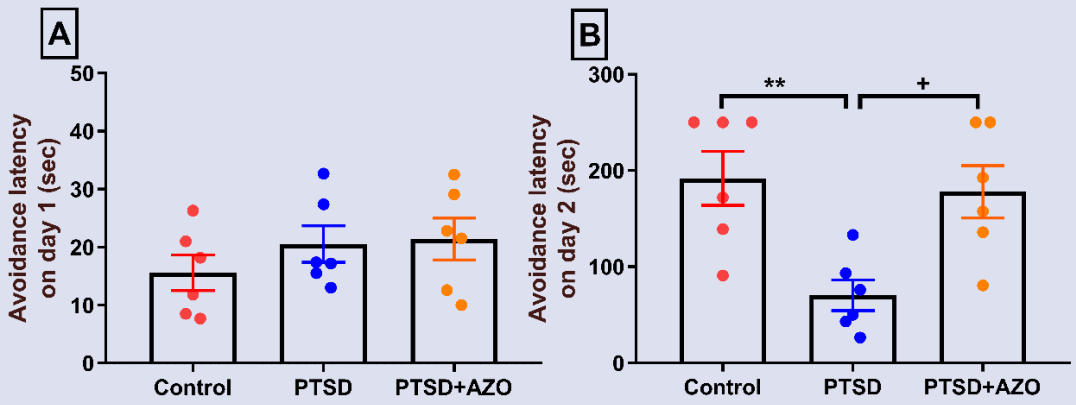

Figure 3. The effect of azosemide treatment on recall behaviors during the acquisition and retention phases of the passive avoidance test in a rat model of PTSD. Data are presented as mean ± standard error of the mean (*p < 0.05, **p < 0.01 compared to the control group; +p < 0.05 compared to the PTSD group; AZO: Azosemide, PTSD: Post-Traumatic Stress Disorder, PTSD + AZO: Post-Traumatic Stress Disorder + Azosemide).

Effect of Azosemide on Hippocampal GABA Levels

The effect of azosemide treatment on hippocampal GABA levels was investigated using the ELISA method in rats subjected to prolonged stress. As shown in Figure 4, hippocampal GABA levels were significantly reduced in both the PTSD and PTSD+AZO groups compared to the control group (Figure 4).

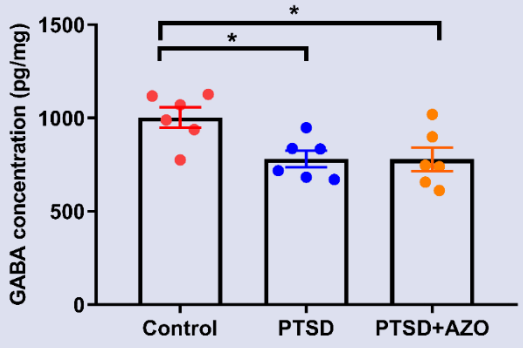

Figure 4. The effect of azosemide treatment on hippocampal GABA levels in the PTSD rat model. The data are presented as mean ± standard error (*p < 0.05 compared to the control group; AZO: Azosemide, PTSD: Post-Traumatic Stress Disorder, PTSD + AZO: Post-Traumatic Stress Disorder + Azosemide).

Overall, our results indicate that azosemide treatment partially improves emotional behavior and significantly improves memory performance in PTSD rats and has no effect on the decrease in hippocampal GABA levels induced by SPS. These results provide insight into the potential therapeutic benefits and limitations of azosemide in PTSD.

Our observation of a partial improvement in emotional behavior suggests that azosemide may modulate specific neurobiological pathways underlying emotional dysregulation in PTSD. The ability of azosemide to cross the blood-brain barrier and inhibit the NKCC1 in the brain may explain its effect on the emotional behavior of rats exposed to SPS. Indeed, it is possible that azosemide may restore impaired chloride homeostasis by inhibiting NKCC1 in the brain, thereby increasing GABAergic inhibition and reducing the symptoms of hyperarousal and anxiety associated with PTSD. It is suggested that proper regulation of chloride homeostasis may help prevent neuropsychiatric disorders due to improper chloride homeostasis and GABA shift abnormalities, such as PTSD [20]. Previous research has implicated dysregulated stress response systems, such as the hypothalamic-pituitary-adrenal (HPA) axis, in the pathophysiology of PTSD [7], [21]. Also, allopregnanolone, a neurosteroid involved in the regulation of the HPA axis, has been reported to be reduced in PTSD and is a potential target for treatment [22]. Thus, the effects of azosemide on brain chloride balance could potentially attenuate some aspects of increased emotional reactivity by indirectly influencing HPA axis activity. However, the partial nature of this improvement suggests that other central mechanisms, such as altered amygdala function or impaired prefrontal-limbic connectivity, remain unaffected by azosemide treatment [21], [23]. In addition, efflux transport across the blood-brain barrier, which limits the penetration of azosemide into the brain, may also play a role in this result [12]. The same researchers reported that systemic administration of NKCC inhibitors such as Azosemide, Torademide and Bumetanide increased the effect of phenobarbital in epileptic mice by crossing the blood brain barrier [16]. Further research is needed to identify these mechanisms and to investigate

whether azosemide treatments that target emotional regulation may be beneficial.

Cognitive deficits, including impaired memory, are hallmarks of PTSD and have been associated with hippocampal dysfunction [24]–[26]. Therefore, our finding that azosemide-treated rats showed improved memory compared with the untreated group is remarkable. It is possible that azosemide improves memory through mechanisms unrelated to hippocampal GABAergic signaling, such as suppressing neuroinflammation by regulating nuclear factor κB (NF-κB) activity, which affects the release of inflammatory factors. It is known that neuroinflammation is positively correlated with memory impairment and that inflammation plays a role in the pathogenesis and pathophysiology of PTSD [27], [28]. Gong et al. reported that inhibition of NKCC1 downregulated inflammatory molecules such as p-p65-NF-κB, IL-6, IL-1β and TNF-α and suppressed neuroinflammation in an surgical brain injury rat model [29]. Although these findings highlight the potential utility of azosemide in improving cognitive impairment in PTSD, the underlying mechanisms require further investigation.

Interestingly, we found that azosemide did not reverse the reduction in hippocampal GABA levels induced by SPS. This finding is consistent with previous evidence suggesting that GABAergic dysfunction in PTSD is driven by complex, multifaceted processes, including not only reduced GABA synthesis, but also altered GABA receptor expression or increased excitatory input [14], [30], [31]. Furthermore, the pharmacological profile of azosemide does not directly target these GABAergic abnormalities, which may explain its lack of effect in restoring hippocampal GABA levels. Indeed, the effect of azosemide on GABAergic activation is indirect in that it reduces the amount of intracellular Cl- ions in neuronal cells, thereby increasing the activity of GABA.

All in all, this study highlights both the therapeutic promise and the limitations of azosemide in the treatment of PTSD. While azosemide appears to be effective in improving memory performance and partially ameliorating emotional disturbance, its inability to restore hippocampal GABA levels highlights the need for multimodal approaches that target the multiple pathophysiological features of PTSD. On the other hand, considering that the application of azosemide treatment for 7 days after the first trauma in our study coincided with the period during which new and permanent brain plasticity related to the stabilization of PTSD occurs in this process, the study points in the direction of elucidating the possible protective role of azosemide treatment in PTSD. In addition, extending this work to include molecular analyses of HPA axis components, inflammatory markers and indicators of oxidative stress may provide a more comprehensive understanding of the mechanisms of action of azosemide in PTSD.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgments

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References

[1] Pai A., Suris AM., North CS, Posttraumatic Stress Disorder in the DSM-5: Controversy, Change, and Conceptual Considerations, *Behav Sci.*, 7(1) (2017) 7.

[2] Schöner J., Heinz A., Endres M., Gertz K., Kronenberg G, Post-traumatic stress disorder and beyond: an overview of rodent stress models, *J Cell Mol Med*., 21(10) (2017) 2248-2256.

[3] Bryant RA, Post-traumatic stress disorder: a state-of-the-art review of evidence and challenges, *World Psychiatry*., 18(3) (2019) 259-269.

[4] Hitchcock C., Goodall B., Sharples O, Population Prevalence of the Posttraumatic Stress Disorder Subtype for Young Children in Nationwide Surveys of the British General Population and of Children in Care, *J Am Acad Child Adolesc Psychiatry*., 60(10) (2021) 1278-1287.

[5] Abdalla S.M., Ettman C.K., Rosenberg, SB, Post-traumatic stress disorder during the Covid-19 pandemic: a national, population-representative, longitudinal study of U.S. adults, *npj Mental Health Res.,* 3 (2024) 20.

[6] Yehuda R., Hoge CW., McFarlane AC, Post-traumatic stress disorder, *Nat Rev Dis Primers*.,1 (2015) 15057

[7] Simeon D., Knutelska M., Yehuda R., Putnam F., Schmeidler J., Smith LM, Hypothalamic-pituitary-adrenal axis function in dissociative disorders, post-traumatic stress disorder, and healthy volunteers, *Biol Psychiatry*., 61(8) (2007) 966-973.

[8] Astill L., Sijbrandij M., Sinnerton R., Lewis C., Roberts N.P., Bisson JI, Pharmacological prevention and early treatment of post-traumatic stress disorder and acute stress disorder: a systematic review and meta-analysis., *Transl Psychiatry*., 9(1) (2019) 334.

[9] Löscher W., Kaila K, CNS pharmacology of NKCC1 inhibitors, *Neuropharmacology*., 205 (2022) 108910.

[10] Suh O.K., Kim S.H., Lee MG, Pharmacokinetics and pharmacodynamics of azosemide, *Biopharm Drug Dispos*., 24(7) (2003) 275-297.

[11] Kaila K., Price T.J., Payne J.A., Puskarjov M., Voipio J, Cation-chloride cotransporters in neuronal development, plasticity and disease, *Nat Rev Neurosci,*. 15(10) (2014) 637-654.

[12] Hampel P., Römermann K., MacAulay N., Löscher W, Azosemide is more potent than bumetanide and various other loop diuretics to inhibit the sodium-potassium-chloride-cotransporter human variants hNKCC1A and hNKCC1B, *Sci Rep*., 8(1) (2018) 9877.

[13] Muñoz A., Méndez P., DeFelipe J., Alvarez-Leefmans FJ, Cation-chloride cotransporters and GABA-ergic innervation in the human epileptic hippocampus, *Epilepsia*., 48(4) (2007) 663-673.

[14] Huang J., Xu F., Yang L, Involvement of the GABAergic system in PTSD and its therapeutic significance, *Front Mol Neurosci*., 20(16) (2023) 1158825.

[15] Souza R.R., Noble L.J., McIntyre CK, Using the Single Prolonged Stress Model to Examine the Pathophysiology of PTSD, *Front Pharmacol*., 8 (2017) 615.

[16] Hampel P., Römermann K., Gailus B, Effects of the NKCC1 inhibitors bumetanide, azosemide, and torasemide alone or in combination with phenobarbital on seizure threshold in epileptic and nonepileptic mice, *Neuropharmacology,* 185 (2021) 108449.

[17] Filiz A.K., Gumus E., Karabulut S., Tastemur Y., Taskiran AS, Protective effects of lamotrigine and vitamin B12 on pentylenetetrazole-induced epileptogenesis in rats, *Epilepsy Behav*., 118 (2021) 107915.

[18] Sahin B., Karabulut S., Filiz AK, Galium aparine L. protects against acetaminophen-induced hepatotoxicity in rats, *Chem Biol Interact*., 366 (2022) 110119.

[19] Yulak F., Joha Z., Öztürk A., İnan D.Ş., Taşkıran AŞ, Enoxaparin Protects C6 Glioma Cells from Glutamate-Induced Cytotoxicity by Reducing Oxidative Stress and Apoptosis, *Mol Neurobiol*., 62(4) (2025) 4631-4640.

[20] Hui K.K., Chater T.E., Goda Y., Tanaka M, How Staying Negative Is Good for the (Adult) Brain: Maintaining Chloride Homeostasis and the GABA-Shift in Neurological Disorders, *Front Mol Neurosci*., 15 (2022) 893111.

[21] Whitaker A.M., Farooq M.A., Edwards S., Gilpin NW, Post-traumatic stress avoidance is attenuated by corticosterone and associated with brain levels of steroid receptor co-activator-1 in rats, *Stress*., 19(1) (2016) 69-77.

[22] Almeida F.B., Pinna G., Barros HMT, The Role of HPA Axis and Allopregnanolone on the Neurobiology of Major Depressive Disorders and PTSD, *Int J Mol Sci*., 22(11) (2021) 5495.

[23] Whitaker A.M., Gilpin N.W., Edwards S, Animal models of post-traumatic stress disorder and recent neurobiological insights, *Behav Pharmacol*. 25(56) (2014) 398-409.

[24] Acheson D.T., Gresack J.E., Risbrough VB, Hippocampal dysfunction effects on context memory: possible etiology for posttraumatic stress disorder, *Neuropharmacology*., 62(2) (2012) 674-685.

[25] Devignes Q., Ren B., Clancy KJ, Trauma-related intrusive memories and anterior hippocampus structural covariance: an ecological momentary assessment study in posttraumatic stress disorder, *Transl Psychiatry,* 14 (2024) 74.

[26] Postel C,. Mary A., Dayan J, Variations in response to trauma and hippocampal subfield changes, *Neurobiol Stress*., 15 (2021) 100346.

[27] Grigoryan GA, Neuroinflammation and Reconsolidation of Memory, *Neurochem. J.,* 16 (2022) 109-120.

[28] Lee D.H., Lee J.Y., Hong DY, Neuroinflammation in Post-Traumatic Stress Disorder, *Biomedicines*., 10(5) (2022) 953.

[29] Gong Y., Wu M., Shen J, Inhibition of the NKCC1/NF-κB Signaling Pathway Decreases Inflammation and Improves Brain Edema and Nerve Cell Apoptosis in an SBI Rat Model, *Front Mol Neurosci*., 14 (2021) 641993.

[30] Kelmendi B., Adams T.G., Yarnell S., Southwick S., Abdallah C.G., Krystal JH, PTSD: from neurobiology to pharmacological treatments, *Eur J Psychotraumatol*., 7 (2016) 31858.

[31] Fang Q., Li Z., Huang GD, Traumatic Stress Produces Distinct Activations of GABAergic and Glutamatergic Neurons in Amygdala, *Front Neurosci*., 12 (2018) 387.