Title: Memantine Alleviates Brain Injury and Neurobehavioral Deficits After Experimental Traumatic Brain Injury in Male Rats

Running Title: Memantine Alleviates Neurobehavioral Deficits

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To appear in: **Basic and Clinical Neuroscience**

Received date: 2022/11/8

Revised date: 2023/03/14

Accepted date: 2023/04/17

Please cite this article as:

Khanian, R., Nazari, S., Nekahi, M., Edalatkhah, S., Farzin, D., Pazhooha, M., et al. (In Press). Memantine Alleviates Brain Injury and Neurobehavioral Deficits After Experimental Traumatic Brain Injury in Male Rats. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.788.2

DOI: http://dx.doi.org/10.32598/bcn.2026.788.2

Abstract

Introduction: Despite its substantial contribution to death and long-term neurological impairment worldwide, effective treatment strategies for traumatic brain injury (TBI) remain scarce. Therefore, this study examined the potential of memantine to reduce brain dysfunction following diffuse experimental TBI.

Materials and Methods: Male rats were randomly assigned to seven groups: intact, sham, vehicle (saline), and three memantine-treated groups receiving 10, 20, or 40 mg/kg. Diffuse TBI was induced, followed by intraperitoneal administration of memantine or vehicle 30 minutes post-injury. Neurological performance was assessed using the veterinary coma scale (VCS). Blood–brain barrier (BBB) disruption was evaluated 4–6 h after traumatic brain injury through Evans Blue extravasation analysis. In parallel, vestibulomotor deficits were monitored for 72 h using beam-balance and beam-walk assessments. Histopathological changes were examined with hematoxylin-eosin staining under light microscopy. Brain water content was determined using the wet/dry method, and cerebrospinal fluid (CSF) concentrations of matrix metalloproteinase-9 (MMP-9), interleukin-1β (IL-1β), and interleukin-10 (IL-10) were quantified by ELISA.

Results: Memantine treatment at 20 and 40 mg/kg significantly attenuated brain edema and BBB disruption while improving neurological performance compared with vehicle-treated animals ($P<0.01$ and $P<0.001$, respectively). CSF MMP-9 levels were markedly reduced in the 40 mg/kg group ($P<0.001$). In addition, these doses favorably regulated inflammatory markers by decreasing IL-1β and increasing IL-10 levels ($P<0.01$ and $P<0.001$, respectively). In contrast, the 10 mg/kg dose showed only modest effects across the measured outcomes.

Conclusion: The present study demonstrates that memantine significantly attenuates TBI-induced neurological deficits, reduces cerebral edema, preserves BBB integrity, and balances pro- and anti-inflammatory cytokines. These findings suggest that memantine may serve as a promising therapeutic strategy for enhancing functional recovery following TBI.

Keywords: Memantine; Neuroprotection; Blood-brain barrier; MMP-9, Interleukins Traumatic brain injury (TBI)

1. Introduction

Among neurological disorders, traumatic brain injury (TBI) constitutes a major source of mortality and long-term disability worldwide, affecting millions of individuals each year. It is estimated that nearly 57 million people are hospitalized annually due to one or more TBIs, although the overall prevalence of TBI-related disability has not been clearly determined (Miller, Daugherty, Waltzman, & Sarmiento, 2021) (Langlois, Rutland-Brown, & Wald, 2006). The World Health Organization (WHO) has estimated that the burden of TBI will increase from 14% to 20% by 2020 (Saatian, Ahmadpoor, Mohammadi, & Mazloumi, 2018). The pathophysiology of TBI is generally described as a two-phase process. The initial insult occurs immediately following mechanical trauma and results in direct structural damage, accompanied by both intracranial and extracranial complications, including hypoxic injury, epidural bleeding, and subarachnoid hemorrhage (Baethmann et al., 1988). Following the primary insult, a cascade of secondary injury mechanisms develops, including excitotoxicity, intracellular calcium overload, blood–brain barrier (BBB) breakdown, and neuroinflammation, collectively contributing to neuronal loss and progressive neurodegeneration (Plesnila, 2016).

Matrix metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes that play a key role in degrading the extracellular matrix. Among them, MMP-9 (also called gelatinase B) is produced by a variety of cell types, including neurons, radial glial cells, endothelial cells, immune cells, and mesenchymal cells (Cunningham, Wetzel, & Rosenberg, 2005). Overexpression of MMPs following brain injury has been linked to increased BBB permeability and subsequent cerebral edema (Rempe, Hartz, Bauer, & Metabolism, 2016).

Memantine is an adamantane derivative with a bridgehead amine (-NH2) that carries a positive charge under physiological conditions (-NH3+) and binds near the Mg2+ site of NMDA receptor-associated channels (Lipton, 2005). By blocking NMDA receptors, memantine is clinically used in the treatment of dementia and Parkinson's disease, and it exhibits neuroprotective effects in models of neural injury such as ischemia and hemorrhage (C. Parsons, Rammes, & Danysz, 2008; Wang, Wee, Hu, Chio, & Kuo, 2018). Studies have demonstrated that memantine promotes neuronal survival, reduces degeneration, and improves gross motor function, despite some minor adverse effects (Day, Carle, & Floyd, 2017). Its neuroprotective potential has been confirmed in numerous in vitro and in vivo experiments. For example, in a rat model of ischemic injury,

administration of memantine two hours post-insult reduced brain damage by roughly 50% (Lipton, 2005). Additionally, memantine protects hippocampal neurons from hypoxic injury (Kelestemur et al., 2016) and has shown efficacy against NMDA-dependent neurotoxicity, hypoxia/ischemia, global forebrain ischemia, and neuropathic pain (Huang et al., 2015).

The present work was designed to assess the neuroprotective effects of memantine after TBI in male rats, particularly on neurological function, brain edema, BBB integrity, and vestibulomotor performance.

2. Materials and Methods

2.1. Animal Model and Experimental Groups

A total of 168 male Wistar rats (250–330 g) were used and maintained under controlled laboratory conditions (22 ± 2°C; 12-h light/dark cycle) with ad libitum access to food and water. All procedures were conducted in accordance with institutional ethical guidelines and approved by the Ethics, Animal Care, and Use Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.RIB.REC.1400.091). Traumatic brain injury (TBI) was induced using Marmarou's weight-drop model (Marmarou, Prieto, Taya, Young, & Marmarou, 2009).

Rats were randomly assigned to seven study groups, with 24 animals in each group:

• Intact group: no intervention was applied.

• Sham group: animals underwent anesthesia and skull exposure without induction of injury.

• TBI group: rats received anesthesia, surgical preparation, and induction of traumatic brain injury.

• Saline group (vehicle): TBI animals received intraperitoneal injection of 0.9% saline 30 min post-injury.

• Memantine-treated groups (10, 20, 40 mg/kg): rats were administered memantine intraperitoneally at the indicated doses 30 min following TBI.

2.2. Diffuse TBI Model

Rats were anesthetized with ketamine and xylazine, followed by endotracheal intubation. A tracheal cannula connected to a ventilatory pump was used to maintain controlled respiration and prevent hypoxic episodes. Diffuse traumatic brain injury was induced according to the Marmarou

weight-drop protocol (Asl, Khaksari, Khachki, Shahrokhi, & Nourizade, 2013; Foda & Marmarou, 1994; Khachaki, Haddad, Shahrokhi, & Sepehri, 2011; Khaksari, Soltani, Shahrokhi, Moshtaghi, & Asadikaram, 2011; Rahimi et al., 2021). Injury severity was evaluated using the Veterinary Coma Scale (VCS), with classifications defined as mild (13–15), moderate (9–12), and severe (3–8). The model did not result in skull fractures, as a sponge cushion placed beneath the head effectively prevented secondary impact damage.

2.3. BBB permeability

Evans blue extravasation was quantified spectrophotometrically as an indicator of BBB permeability 4 h after TBI following systemic administration of 2% Evans blue (20 mg/kg) via the jugular vein (n = 8/group). After anesthesia with thiopental (50 mg/kg, i.p.), animals underwent transcardial perfusion with heparinized saline to eliminate intravascular dye. Brains were harvested, weighed, homogenized, and incubated in acetone-based solution for 24 h. Following centrifugation, absorbance of the supernatant was measured at 620 nm and used to determine Evans blue content in brain tissue (Khachaki et al., 2011; Rahimi et al., 2021).

2.4. Brain Water Content

At 24 h after TBI, brain tissues were collected and immediately weighed to determine wet weight (WW). Samples were then dried at 60–70°C for 72 h to obtain dry weight (DW). Brain water content was calculated using the wet–dry weight method based on the difference between wet and dry weights relative to wet weight (Khaksari, Maghool, Asadikaram, & Hajializadeh, 2016).

2.5. Neurological Evaluation

Neurological function was assessed using VCS (score range 3–15) encompassing motor, eye, and respiratory components. Higher scores indicate better neurological outcomes. Measurements were taken pre-TBI, immediately after TBI (D0), and at 24 h (D1), 48 h (D2), and 72 h (D3) post-TBI (n = 8/group) (Rahimi, Ferdowsi, & Siahposht-Khachaki, 2020; Soltani et al., 2015).

2.6. Vestibulomotor function

The beam task assessed motor balance. Rats were trained before TBI: five trials on a 100 cm × 4 cm beam, and five trials on a 100 cm × 1.5 cm beam. On testing day, three trials were averaged

per animal. Performance was recorded with a camera, and time to traverse or balance was scored, with a maximum of 5 points for remaining 60 seconds on the beam (Monaco et al., 2013; Rahimi et al., 2021).

2.7. CSF Collection and ELISA

CSF samples were obtained 72 hours post-TBI (n = 8/group) via cisterna magna puncture under local anesthesia (0.25 mL 1% lidocaine) using a 25-gauge syringe. Collected CSF (50–100 µL) was centrifuged at 1,000 × g for 15 min and stored at −80°C. MMP-9, IL-1β, and IL-10 levels were quantified using rat ELISA kits (MBS722532, MyBioSource, San Diego, CA, USA) (Nirogi et al., 2009; Pegg, He, Stroink, Kattner, & Wang, 2010).

2.8. Histopathology

At 72 h after TBI, two animals from each group were anesthetized with thiopental (50 mg/kg, i.p.), sacrificed, and their brains were harvested and fixed in 10% buffered formalin. Tissue samples were sectioned at 5 µm using an automatic microtome (LEICA, Germany) and stained with hematoxylin and eosin. Histopathological alterations were then examined under light microscopy by two blinded pathologists (Meymandi et al., 2018; Rahimi et al., 2021).

2.9. Statistical Analysis

Results are expressed as mean ± SEM. Data normality was evaluated using the Shapiro–Wilk test. For parametric data, one-way ANOVA followed by Tukey's post hoc test was applied, while non-parametric variables were analyzed using the Kruskal–Wallis test with Dunn's multiple comparisons. A significance threshold of $P < 0.05$ was considered. All analyses and graphical presentations were performed using GraphPad Prism version 8.0 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Neurological Function (VCS)

Baseline VCS scores did not differ among the experimental groups prior to injury induction. Following TBI, a marked decline in neurological performance was observed across all injured groups compared with the sham and intact animals (Fig. 1A) ($p < 0.001$). At 24 hours post-injury,

administration of memantine at 40 mg/kg significantly improved VCS scores relative to the TBI and saline-treated groups ($p < 0.01$). By 48 hours, both 20 and 40 mg/kg doses produced significant neurological recovery compared with untreated TBI animals ($p < 0.01$). Notably, at 48 and 72 hours, animals receiving 10 mg/kg did not show significant differences compared with the sham or intact groups. Seventy-two hours after trauma, treatment with 20 and 40 mg/kg memantine continued to demonstrate significant improvement over the TBI and saline groups ($p < 0.01$ and $p < 0.05$, respectively). Furthermore, comparison among treatment doses revealed superior recovery in the 40 mg/kg group compared with 20 mg/kg at both 48 and 72 hours ($p < 0.01$). Assessment of the area under the curve (AUC) confirmed these findings (Fig. 1B). All injured groups differed significantly from sham and intact animals ($p < 0.01$), while 20 and 40 mg/kg memantine significantly increased AUC values relative to TBI and saline groups ($p < 0.01$ and $p < 0.001$, respectively).

3.2. Vestibulomotor Performance (Beam-Walk and Beam-Balance)

The present work was designed to assess the neuroprotective effects of memantine after TBI in male rats, particularly on neurological function, brain edema, BBB integrity, and vestibulomotor performance. (Fig. 2A) ($p < 0.001$), and this impairment persisted at 24 hours. At 48 hours post-TBI, rats treated with 20 or 40 mg/kg memantine exhibited significant improvement in beam-walk performance relative to TBI and saline groups ($p < 0.01$ and $p < 0.001$, respectively). This functional recovery remained evident through the final assessment time point. Seventy-two hours after injury, no significant difference was observed between the 10 mg/kg group and the sham or intact animals. AUC analysis yielded parallel outcomes, with the 20 and 40 mg/kg groups demonstrating significantly lower cumulative impairment compared with TBI animals (Fig. 2B) ($p < 0.01$ and $p < 0.001$, respectively). The 40 mg/kg dose showed the most pronounced effect. Beam-balance testing produced consistent results (Fig. 2C). Significant functional improvement was detected at 48 and 72 hours in the 20 and 40 mg/kg groups compared with TBI and saline animals ($p < 0.01$ and $p < 0.001$). The 10 mg/kg dose failed to elicit a measurable effect. AUC analysis showed no detectable differences between this group and the TBI, saline, sham, or intact groups (Fig. 2D) ($p > 0.05$).

3.3. Brain Water Content

At 24 h after TBI, brain water content was markedly increased in both the TBI and saline-treated groups compared with sham and intact controls ($p < 0.001$), reflecting significant edema formation (Fig. 3). Administration of memantine at 40 mg/kg significantly reduced brain water content relative to the TBI and saline groups ($p < 0.001$). The 20 mg/kg dose showed a decreasing trend in edema compared with the TBI group, although this effect was not statistically significant versus saline. In contrast, treatment with 10 mg/kg memantine had no meaningful impact on brain water content.

3.4. Blood–Brain Barrier Integrity

Evans blue extravasation demonstrated substantial BBB disruption in TBI and saline groups compared with sham and intact animals (Fig. 4) ($p < 0.001$). Administration of memantine at 40 mg/kg significantly reduced dye accumulation in brain tissue relative to TBI and saline groups ($p < 0.001$), suggesting preservation of BBB integrity. No significant protective effect was observed with the 10 mg/kg dose.

3.5. CSF MMP-9 Levels

TBI markedly elevated MMP-9 concentrations in CSF compared with sham and intact animals (Fig. 5) ($p < 0.001$). Treatment with memantine at 20 and 40 mg/kg effectively reduced MMP-9 levels relative to TBI and saline groups ($p < 0.001$), with a more pronounced effect observed at 40 mg/kg. In contrast, the 10 mg/kg dose had no measurable impact on MMP-9 levels.

3.6. Pro- and Anti-Inflammatory Cytokines

Significant alterations in cytokine levels were observed following TBI. IL-1β levels increased markedly in the TBI group compared with sham and intact controls (Fig. 6A) ($p < 0.001$). Memantine at 20 and 40 mg/kg significantly reduced IL-1β concentrations. Conversely, IL-10 levels were significantly elevated in these treatment groups, indicating enhanced anti-inflammatory response (Fig. 6B). The 10 mg/kg dose did not significantly affect IL-1β or IL-10 levels.

3.7. Histopathological Findings

Microscopic evaluation demonstrated normal cortical organization in the intact and sham groups, with well-preserved neuronal morphology. Neurons exhibited basophilic, euchromatic, oval-shaped soma, and astrocytes showed normal structure and distribution (Fig. 7a, b). In contrast, the TBI and saline groups exhibited pronounced histopathological damage characterized by neuronal edema and structural disorganization, with darkly stained (pyknotic) nuclei, along with perivascular edema, neuronal necrosis, endothelial swelling, and vascular congestion (Fig. 7c, d). No noticeable histological difference was detected between these two groups. Treatment with memantine at 20 mg/kg attenuated tissue damage, as evidenced by a reduced number of degenerated and edematous neurons compared with untreated TBI animals (Fig. 7f). More pronounced neuroprotection was observed in the 40 mg/kg memantine group. Most neurons maintained normal morphology with euchromatic nuclei and distinct nucleoli. Vascular structures, endothelial cells, and astrocytes appeared comparable to those seen in intact and sham groups (Fig. 7g). By comparison, rats receiving 10 mg/kg memantine exhibited marked histopathological alterations comparable to those seen in the TBI and vehicle groups (Fig. 7e).

4. Discussion

In the present study, the neuroprotective potential of memantine was evaluated in a rat model of traumatic brain injury using a range of functional, biochemical, and pathological endpoints, including neurological status (VCS), vestibulomotor performance, brain edema, blood–brain barrier (BBB) integrity, and CSF concentrations of inflammatory mediators and MMP-9. The results showed that post-traumatic administration of memantine improved neurological and motor outcomes, alleviated cerebral edema, preserved BBB function, reduced IL-1β levels, and enhanced IL-10 concentrations relative to vehicle-treated animals. These beneficial effects were most pronounced at doses of 20 and 40 mg/kg, which significantly increased VCS scores by day 3 after injury and promoted recovery in beam-walk and beam-balance assessments. In contrast, treatment with 10 mg/kg produced only limited improvement in vestibulomotor function.

Traumatic brain injury remains a major cause of morbidity and mortality worldwide (Sabet, Soltani, & Khaksari, 2021). Although both amantadine and memantine are commonly used in acute post-injury rehabilitation, they currently lack formal FDA approval for neurorehabilitation

purposes (H. M. Ma & Zafonte, 2020). Preclinical studies suggest that early administration of memantine can improve TBI outcomes by modulating NR2B subunit expression, reducing neuronal apoptosis, and diminishing nitrosative stress in damaged cortical tissue (Wang et al., 2018). Furthermore, memantine provides neuroprotection against white matter injury, demyelination, and oligodendrocyte loss following repetitive mild TBI (G. Ma et al., 2019). As a low-affinity, non-competitive NMDA receptor antagonist, memantine exhibits neuroprotective properties across multiple injury models, including cerebral and spinal cord ischemia as well as TBI (C. Parsons et al., 2008). Studies have also indicated that blocking NMDAR function with antagonists such as amantadine improves cognitive outcomes after mild TBI (H. M. Ma & Zafonte, 2020).

The pharmacological profile of memantine allows it to act more effectively and with less voltage dependence than Mg^{2+}, and its monovalent charge may render it a particularly effective substitute for Mg^{2+} (C. G. Parsons, Gruner, Rozental, Millar, & Lodge, 1993). Its mechanism involves a partial blockade of NMDA receptor-mediated excitotoxicity, permitting neuronal survival while preserving normal synaptic transmission. Preclinical investigations have shown that memantine prevents ischemia-induced memory deficits and mitigates neurological damage following cerebral ischemia (Watanabe et al., 2010). Its neuroprotective effects extend to cerebrocortical neurons, cerebellar neurons, and retinal neurons (Lipton, 2006). Even brief exposure of cultured cortical neurons to memantine can reduce NMDA receptor-mediated toxicity (Tremblay et al., 2000), and immediate post-injury administration has been shown to prevent neuronal loss in hippocampal regions in TBI models (Volbracht, Van Beek, Zhu, Blomgren, & Leist, 2006). Clinically, memantine has been associated with reductions in serum neuron-specific enolase (NSE) and improvements in the Glasgow Coma Scale (GCS) among TBI patients. NSE is a well-established biomarker of neuronal injury, as its release into extracellular space and bloodstream following neural damage correlates with worse outcomes (Khan, Ali, Kadir, Ahmed, & Di Pietro, 2021). Additionally, memantine significantly diminishes lipid peroxidation in rat TBI models (Özsüer, Görgülü, Kırış, & Çobanoğlu, 2005) and improves GCS scores over the first three days post-injury (Welling, Welling, & Figueiredo, 2018).

Memantine has been shown to attenuate cerebral infarction and neuronal injury following ischemia, while also reducing cortical neuronal apoptosis induced by oxygen–glucose deprivation.

In addition, it decreases the number of damaged astrocytes and excessively activated microglia within 24 h after ischemic injury (Chen et al., 2016). Increased endothelial permeability and subsequent BBB impairment are central to the pathological cascade in brain ischemia and secondary reperfusion injury. Evidence indicates that memantine prevents ischemia–reperfusion (I/R)-induced upregulation of tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) at both mRNA and protein levels. In cerebral ischemia, matrix metalloproteinases (MMPs) have dual roles: acutely they disrupt tight junction proteins (TJPs) in the BBB, and chronically they promote angiogenesis. Memantine has been shown to reduce endothelial monolayer permeability after I/R by increasing expression of TJPs such as occludin and the adherens junction protein VE-cadherin. Moreover, memantine decreases expression and activity of MMP-2 but not MMP-9 following I/R (Liu, Huang, Xu, Qu, & Wang, 2018).

In experimental subarachnoid hemorrhage (SAH), memantine preserves BBB integrity, rescues neuronal damage, and improves neurological outcomes. Consistent with these findings, our study revealed that memantine inhibits MMP-9 expression and activity. MMP expression and activity are regulated at multiple levels, including transcriptional, translational, and post-translational control. Memantine treatment has been demonstrated to prevent MMP-2 expression at both mRNA and protein levels (Gürsoy-Özdemir, Can, & Dalkara, 2004). BBB disruption initiates a cycle of injury, edema formation, and disturbed intracranial hemodynamics, resulting in further ischemia. Several studies suggest that NMDA antagonists may mitigate BBB damage in SAH and other acute CNS insults (Germanò et al., 2007). Additionally, memantine reduces BBB breakdown after cerebral ischemia (Görgülü, Kınş, Çobanoğlu, Yanık, & Küçük, 2000). and experimental encephalomyelitis (Paul & Bolton, 2002). Memantine also attenuates cell death, astrogliosis, and functional deficits in brain trauma models (Effgen & Morrison III, 2017).

Our measurements of pro- and anti-inflammatory cytokines in CSF 24 hours after TBI indicated that Treatment with memantine at 20 and 40 mg/kg significantly reduced IL-1β and increased IL-10, demonstrating a reduction in neuroinflammatory response. Finally, assessment of MMP-9 in CSF 72 hours post-TBI showed that memantine at these doses effectively reduced MMP-9 levels, corroborating previous findings regarding the temporal profile of MMP-9 elevation in CSF following brain injury (Hadass et al., 2013; Wu et al., 2020). These results collectively support the dual mechanism of memantine: mitigating excitotoxicity while modulating inflammatory

pathways, thereby contributing to observed improvements in both neurological function and histopathology.

Conclusion

Overall, our findings suggest that memantine provides substantial protection against TBI-induced brain injury. Memantine administration improved neurological function, brain water content (BW), and vestibulomotor performance, while reducing cerebral edema, BBB permeability, and neuroinflammation.

Abbreviations: TBI: Traumatic Brain Injury; BBB: Blood Brain Barrier; BW: Beam-Walk; BB: Beam-Balance; CSF: Cerebrospinal fluid; VCS: Veterinary Coma Scale; AUC: Area under the Curves; TNFα: Tumor Necrosis Factor alpha; MMP-9: Matrix Metalloproteinase-9.

References

Asl, S. Z., Khaksari, M., Khachki, A. S., Shahrokhi, N., & Nourizade, S. (2013). Contribution of estrogen receptors alpha and beta in the brain response to traumatic brain injury. *Journal of neurosurgery, 119*(2), 353-361.

Baethmann, A., Maier-Hauff, K., Kempski, O., Unterberg, A., Wahl, M., & Schürer, L. J. C. c. m. (1988). Mediators of brain edema and secondary brain damage. *16*(10), 972-978.

Chen, Z.-Z., Yang, D.-D., Zhao, Z., Yan, H., Ji, J., & Sun, X.-L. (2016). Memantine mediates neuroprotection via regulating neurovascular unit in a mouse model of focal cerebral ischemia. *Life sciences, 150*, 8-14.

Cunningham, L. A., Wetzel, M., & Rosenberg, G. A. J. G. (2005). Multiple roles for MMPs and TIMPs in cerebral ischemia. *50*(4), 329-339.

Day, N. L., Carle, M. S., & Floyd, C. L. (2017). Post-injury administration of a combination of memantine and 17β-estradiol is protective in a rat model of traumatic brain injury. *Neurochemistry international, 111*, 57-68.

Effgen, G. B., & Morrison III, B. (2017). Memantine reduced cell death, astrogliosis, and functional deficits in an in vitro model of repetitive mild traumatic brain injury. *Journal of neurotrauma, 34*(4), 934-942.

Foda, M. A. A.-E., & Marmarou, A. J. J. o. n. (1994). A new model of diffuse brain injury in rats: Part II: Morphological characterization. *80*(2), 301-313.

Germanò, A., Caffo, M., Angileri, F. F., Arcadi, F., Newcomb-Fernandez, J., Caruso, G., . . . Wang, K. K. (2007). NMDA receptor antagonist felbamate reduces behavioral deficits and blood–brain barrier permeability changes after experimental subarachnoid hemorrhage in the rat. *Journal of neurotrauma, 24*(4), 732-744.

Görgülü, A., Kınş, T., Çobanoğlu, S., Yanık, B., & Küçük, M. (2000). Reduction of edema and infarction by Memantine and MK-801 after focal cerebral ischaemia and reperfusion in rat. *Acta neurochirurgica, 142*(11), 1287-1292.

Gürsoy-Özdemir, Y., Can, A., & Dalkara, T. (2004). Reperfusion-induced oxidative/nitrative injury to neurovascular unit after focal cerebral ischemia. *Stroke, 35*(6), 1449-1453.

Hadass, O., Tomlinson, B. N., Gooyit, M., Chen, S., Purdy, J. J., Walker, J. M., . . . Gu, Z. (2013). Selective Inhibition of Matrix Metalloproteinase-9 Attenuates Secondary Damage Resulting from Severe Traumatic Brain Injury. *PLOS ONE, 8*(10), e76904. doi:10.1371/journal.pone.0076904

Huang, C.-Y., Wang, L.-C., Wang, H.-K., Pan, C.-H., Cheng, Y.-Y., Shan, Y.-S., . . . Tsai, K.-J. (2015). Memantine alleviates brain injury and neurobehavioral deficits after experimental subarachnoid hemorrhage. *Molecular neurobiology, 51*(3), 1038-1052.

Kelestemur, T., Yulug, B., Caglayan, A. B., Beker, M. C., Kilic, U., Caglayan, B., . . . Kilic, E. (2016). Targeting different pathophysiological events after traumatic brain injury in mice: Role of melatonin and memantine. *Neuroscience letters, 612*, 92-97.

Khachaki, A. S., Haddad, M. K., Shahrokhi, N., & Sepehri, G. (2011). Effects of different phases of estrous cycle on brain edema and neurological outcomes after severe traumatic brain injury in female rats. *Koomesh*, *13*(1).

Khaksari, M., Maghool, F., Asadikaram, G., & Hajializadeh, Z. (2016). Effects of sex steroid hormones on neuromedin S and neuromedin U2 receptor expression following experimental traumatic brain injury. *Iranian journal of basic medical sciences*, *19*(10), 1080.

Khaksari, M., Soltani, Z., Shahrokhi, N., Moshtaghi, G., & Asadikaram, G. (2011). The role of estrogen and progesterone, administered alone and in combination, in modulating cytokine concentration following traumatic brain injury. *Canadian journal of physiology and pharmacology*, *89*(1), 31-40.

Khan, S., Ali, A., Kadir, B., Ahmed, Z., & Di Pietro, V. (2021). Effects of memantine in patients with traumatic brain injury. *recall*, *9*, 13.

Langlois, J. A., Rutland-Brown, W., & Wald, M. M. J. T. J. o. h. t. r. (2006). The epidemiology and impact of traumatic brain injury: a brief overview. *21*(5), 375-378.

Lipton, S. A. (2005). The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. *Current Alzheimer Research*, *2*(2), 155-165.

Lipton, S. A. (2006). Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nature reviews Drug discovery*, *5*(2), 160-170.

Liu, Y., Huang, Y., Xu, Y., Qu, P., & Wang, M. (2018). Memantine protects against ischemia/reperfusion-induced brain endothelial permeability. *IUBMB life*, *70*(4), 336-343.

Ma, G., Liu, C., Hashim, J., Conley, G., Morriss, N., Meehan, W. P., . . . Mannix, R. (2019). Memantine mitigates oligodendrocyte damage after repetitive mild traumatic brain injury. *Neuroscience*, *421*, 152-161.

Ma, H. M., & Zafonte, R. D. (2020). Amantadine and memantine: a comprehensive review for acquired brain injury. *Brain Injury*, *34*(3), 299-315.

Marmarou, C., Prieto, R., Taya, K., Young, H., & Marmarou, A. (2009). Marmarou Weight Drop Injury Model. In (pp. 393-407).

Meymandi, M. S., Soltani, Z., Sepehri, G., Amiresmaili, S., Farahani, F., & Aghtaei, M. M. (2018). Effects of pregabalin on brain edema, neurologic and histologic outcomes in experimental traumatic brain injury. *Brain research bulletin*, *140*, 169-175.

Miller, G. F., Daugherty, J., Waltzman, D., & Sarmiento, K. (2021). Predictors of traumatic brain injury morbidity and mortality: examination of data from the national trauma data bank: predictors of TBI morbidity & mortality. *Injury*, *52*(5), 1138-1144.

Monaco, C. M., Mattiola, V. V., Folweiler, K. A., Tay, J. K., Yelleswarapu, N. K., Curatolo, L. M., . . . Kline, A. E. (2013). Environmental enrichment promotes robust functional and histological benefits in female rats after controlled cortical impact injury. *Experimental neurology*, *247*, 410-418.

Nirogi, R., Kandikere, V., Mudigonda, K., Bhyrapuneni, G., Muddana, N., Saralaya, R., & Benade, V. (2009). A simple and rapid method to collect the cerebrospinal fluid of rats and its

application for the assessment of drug penetration into the central nervous system. *Journal of neuroscience methods, 178*(1), 116-119.

Özsüer, H., Görgülü, A., Kırış, T., & Çobanoğlu, S. (2005). The effects of memantine on lipid peroxidation following closed-head trauma in rats. *Neurosurgical review, 28*(2), 143-147.

Parsons, C., Rammes, G., & Danysz, W. (2008). Pharmacodynamics of memantine: an update. *Current neuropharmacology, 6*(1), 55-78.

Parsons, C. G., Gruner, R., Rozental, J., Millar, J., & Lodge, D. (1993). Patch clamp studies on the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3, 5-dimethyladamantan). *Neuropharmacology, 32*(12), 1337-1350.

Paul, C., & Bolton, C. (2002). Modulation of blood-brain barrier dysfunction and neurological deficits during acute experimental allergic encephalomyelitis by then-methyl-d-aspartate receptor antagonist memantine. *Journal of Pharmacology and Experimental Therapeutics, 302*(1), 50-57.

Pegg, C. C., He, C., Stroink, A. R., Kattner, K. A., & Wang, C. X. (2010). Technique for collection of cerebrospinal fluid from the cisterna magna in rat. *Journal of neuroscience methods, 187*(1), 8-12.

Plesnila, N. J. C. o. i. p. (2016). The immune system in traumatic brain injury. *26*, 110-117.

Rahimi, S., Dadfar, B., Tavakolian, G., Rad, A. A., Shabkahi, A. R., & Siahposht-Khachaki, A. (2021). Morphine attenuates neuroinflammation and blood-brain barrier disruption following traumatic brain injury through the opioidergic system. *Brain Research Bulletin, 176*, 103-111.

Rahimi, S., Ferdowsi, A., & Siahposht-Khachaki, A. J. M. B. D. (2020). Neuroprotective effects of metformin on traumatic brain injury in rats is associated with the AMP-activated protein kinase signaling pathway. *35*(7), 1135-1144.

Rempe, R. G., Hartz, A. M., Bauer, B. J. J. o. C. B. F., & Metabolism. (2016). Matrix metalloproteinases in the brain and blood–brain barrier: versatile breakers and makers. *36*(9), 1481-1507.

Saatian, M., Ahmadpoor, J., Mohammadi, Y., & Mazloumi, E. (2018). Epidemiology and pattern of traumatic brain injury in a developing country regional trauma center. *Bulletin of Emergency & Trauma, 6*(1), 45.

Soltani, Z., Khaksari, M., Jafari, E., Iranpour, M., Shahrokhi, N. J. P., & Behavior. (2015). Is genistein neuroprotective in traumatic brain injury? *, 152*, 26-31.

Tremblay, R., Chakravarthy, B., Hewitt, K., Tauskela, J., Morley, P., Atkinson, T., & Durkin, J. P. (2000). Transient NMDA receptor inactivation provides long-term protection to cultured cortical neurons from a variety of death signals. *Journal of Neuroscience, 20*(19), 7183-7192.

Volbracht, C., Van Beek, J., Zhu, C., Blomgren, K., & Leist, M. (2006). Neuroprotective properties of memantine in different in vitro and in vivo models of excitotoxicity. *European Journal of Neuroscience, 23*(10), 2611-2622.

Wang, C.-C., Wee, H.-Y., Hu, C.-Y., Chio, C.-C., & Kuo, J.-R. (2018). The Effects of Memantine on Glutamic Receptor–Associated Nitrosative Stress in a Traumatic Brain Injury Rat Model. *World neurosurgery, 112*, e719-e731.

Watanabe, T., Iwasaki, K., Takasaki, K., Yamagata, N., Fujino, M., Nogami, A., . . . Fujiwara, M. (2010). Dynamin 1 depletion and memory deficits in rats treated with Aβ and cerebral ischemia. *Journal of neuroscience research, 88*(9), 1908-1917.

Welling, L. C., Welling, M. S., & Figueiredo, E. G. (2018). Memantine: From Alzheimer Disease to Traumatic Brain Injury. *World neurosurgery*, *122*, 293-293.

Wu, M.-Y., Gao, F., Yang, X.-M., Qin, X., Chen, G.-Z., Li, D., . . . Chen, G. (2020). Matrix metalloproteinase-9 regulates the blood brain barrier via the hedgehog pathway in a rat model of traumatic brain injury. *Brain Research, 1727*, 146553. doi:https://doi.org/10.1016/j.brainres.2019.146553

Figure legends

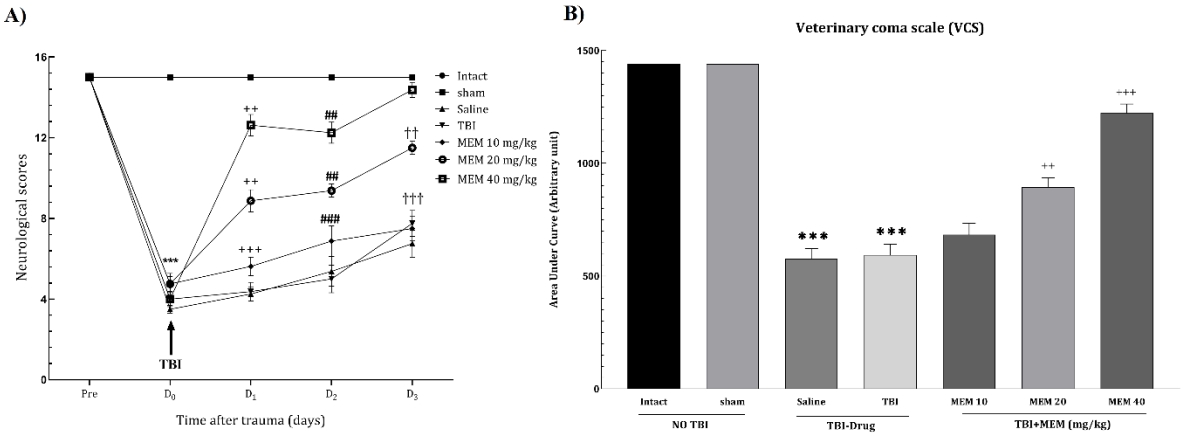

Fig. 1. (A) VCS scores of all experimental groups measured over three consecutive days following TBI (n = 7 per group). Data points represent mean ± SEM. Statistical analysis was performed using repeated-measures two-way ANOVA with Greenhouse-Geisser correction, followed by Tukey's HSD for post-hoc comparisons. (B) Area under the curve (AUC) of VCS scores. One-way ANOVA was used for statistical analysis with Newman-Keuls post-hoc test.

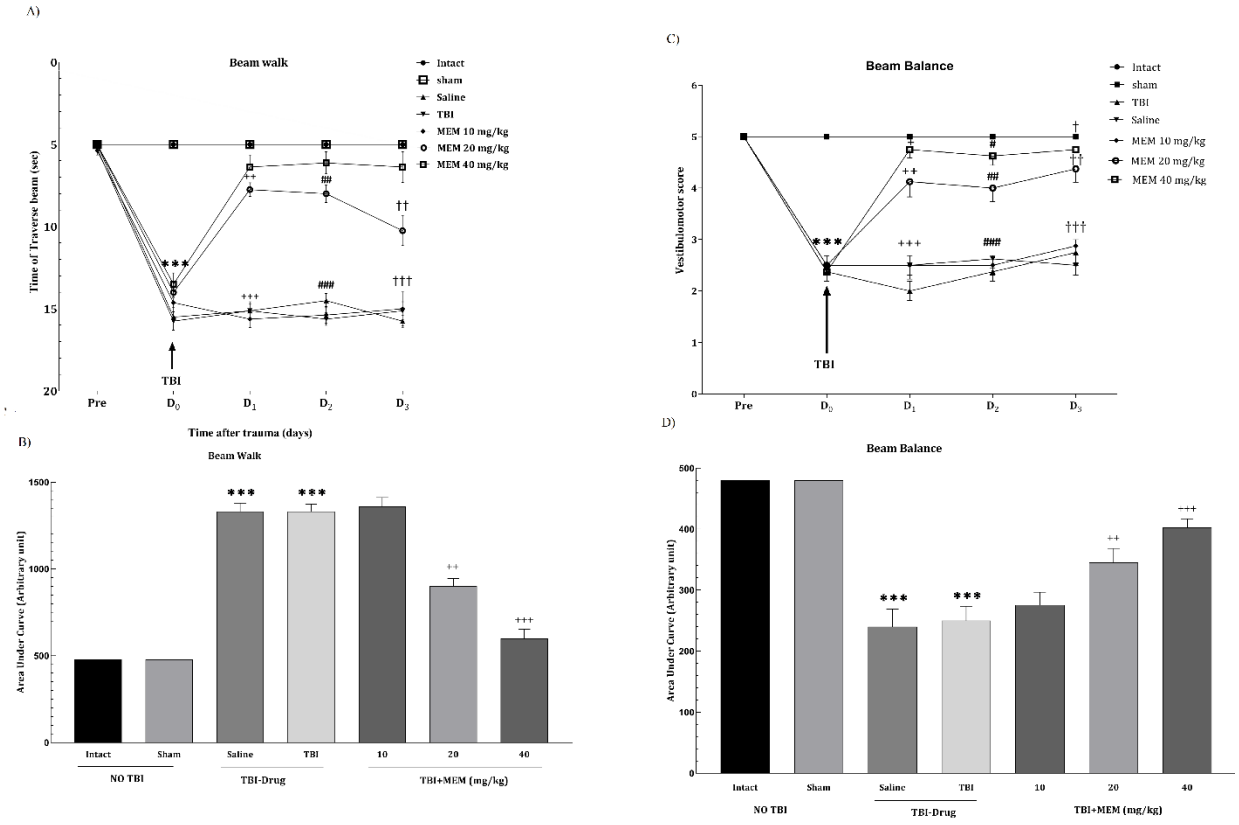

Fig. 2. Beam-walk and beam-balance task performance over three consecutive days following TBI (n = 8 per group). Data points represent mean ± SEM. (A) Beam-walk task: time to traverse the beam (seconds) over three days post-injury. Repeated-measures two-way ANOVA with Greenhouse-Geisser correction was used, followed by Tukey's HSD post-hoc test. (B) Area under the curve (AUC) for beam-walk task, analyzed by one-way ANOVA with Newman-Keuls post-hoc test. (C) Beam-balance task: time to maintain balance on the beam (seconds) over three days post-injury. Statistical analysis performed using repeated-measures two-way ANOVA with Greenhouse-Geisser correction and Tukey's HSD post-hoc test. (D) AUC for beam-balance task, analyzed by one-way ANOVA with Newman-Keuls post-hoc test.

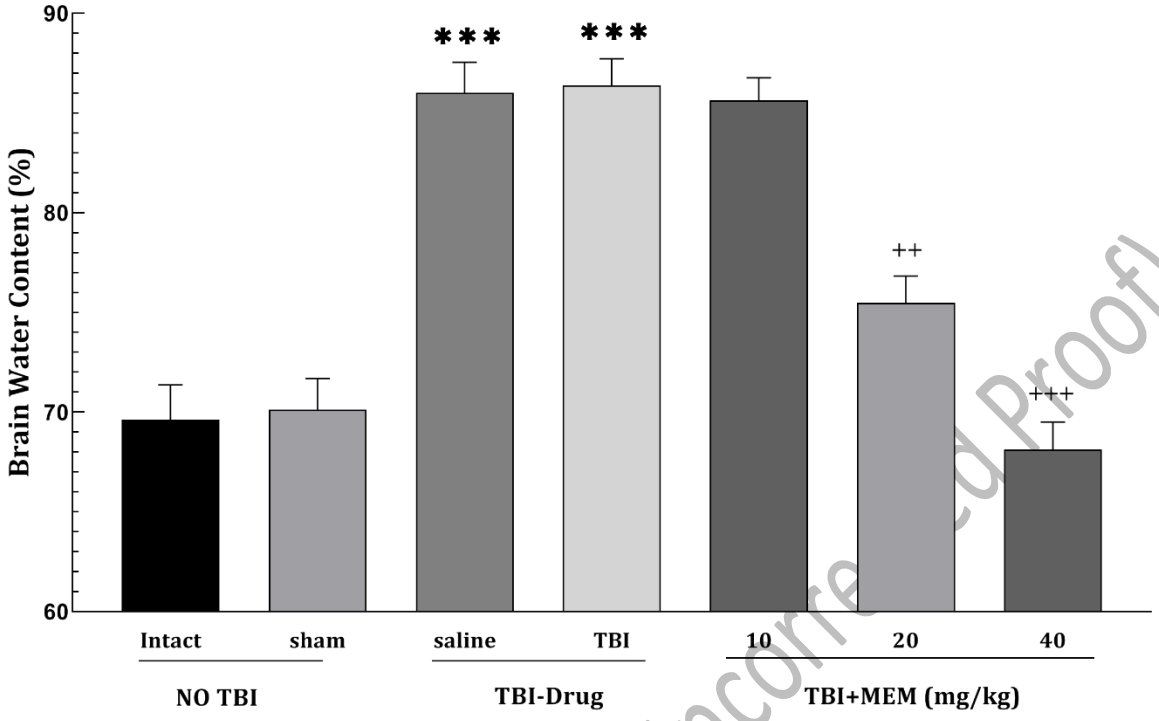

Fig. 3. Effect of memantine (MEM) on brain water content (%) 24 hours after TBI. Bars represent mean ± SEM (n = 8 per group). Statistical analysis was performed using one-way ANOVA followed by Newman-Keuls post-hoc test.

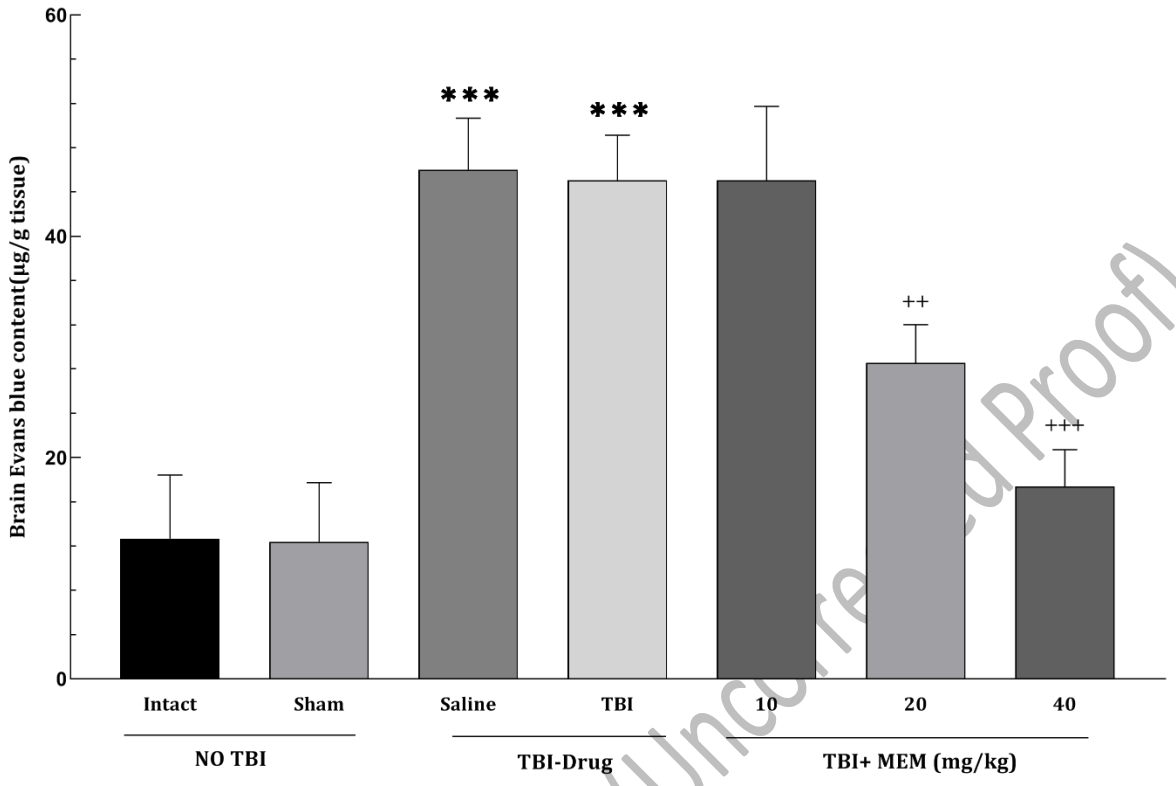

Fig. 4. Effect of memantine (MEM) on Evans blue dye content in brain tissue 6 hours after TBI. Bars represent mean ± SEM (n = 8 per group). Statistical comparisons were performed using one-way ANOVA followed by Newman-Keuls post-hoc test.

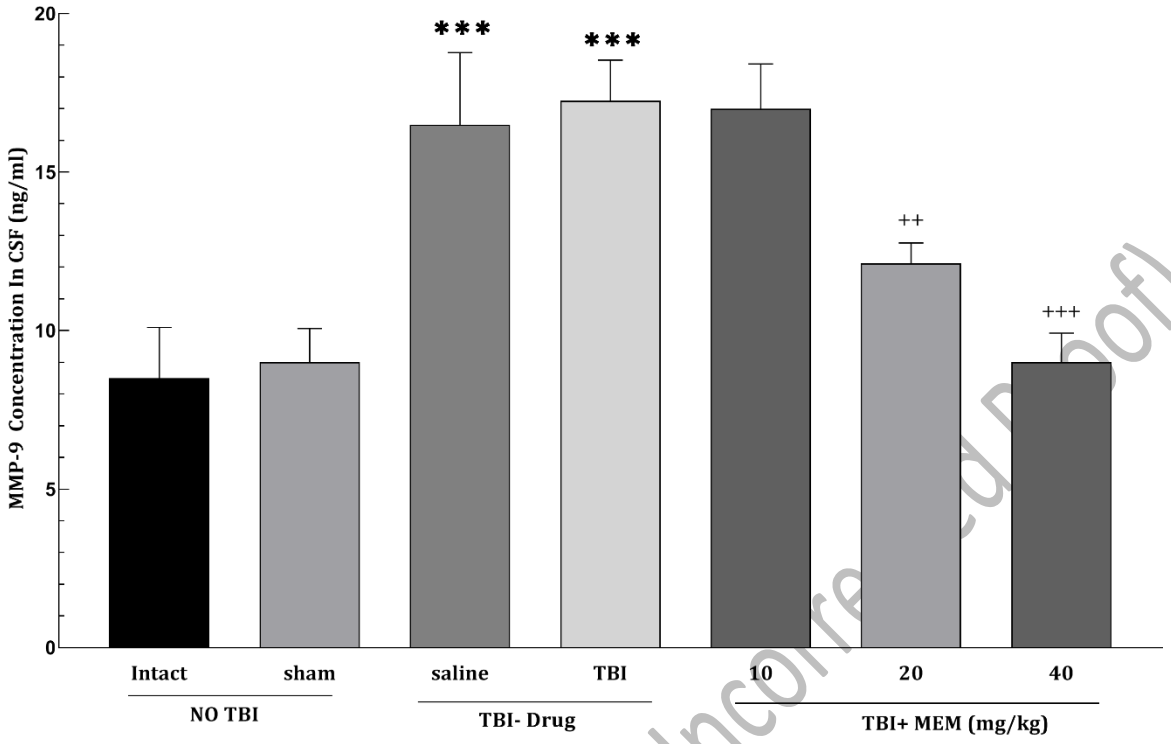

Fig. 5. Cerebrospinal fluid (CSF) levels of MMP-9 measured by ELISA 24 hours after TBI (n = 8 per group). Bars represent mean ± SEM. Statistical analysis was performed using one-way ANOVA with Newman-Keuls post-hoc test.

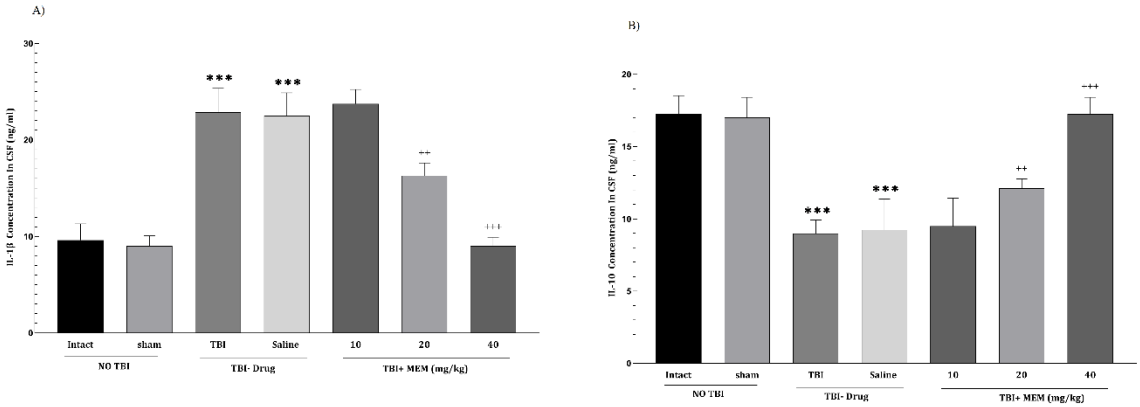

Fig. 6. Effects of memantine on CSF levels of IL-1β and IL-10 at 24 h after TBI (n = 8 per group). Bars represent mean ± SEM. Data were analyzed using the Kruskal-Wallis test followed by Dunn's post-hoc multiple comparison test. (A) IL-1β levels in CSF across groups. (B) IL-10 levels in CSF across groups.

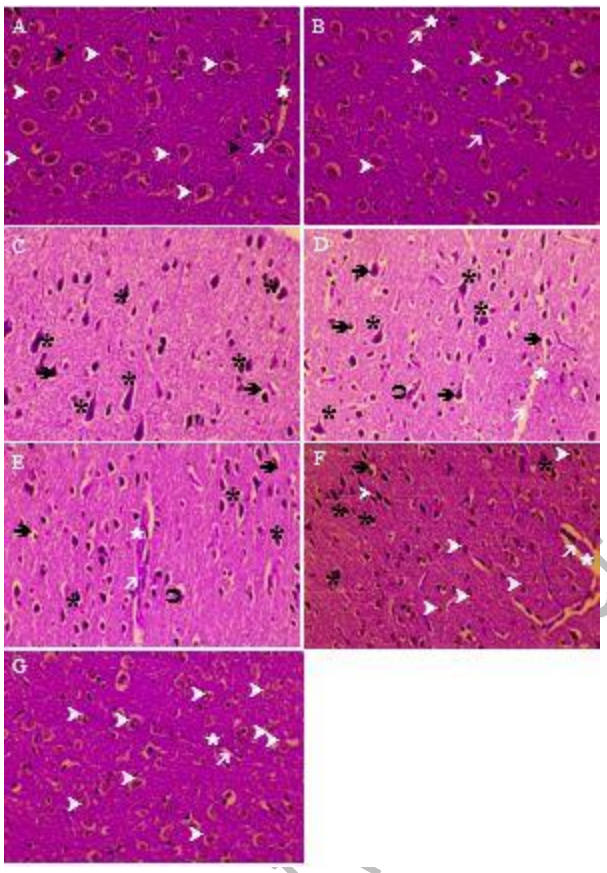

Fig. 7. Histopathological evaluation of rat brains 24 h after TBI at 400× magnification. Groups are as follows: (A) intact, (B) sham, (C) TBI, (D) saline, (E) MEM 10 mg/kg, (F) MEM 20 mg/kg, (G) MEM 40 mg/kg. Symbols indicate:

➢(white): Normal neuron, * : Degenerated neuron, ➔: Edematous neuron, ➲:Swollen Astrocyte, ★: Blood Vessel, ↗(white) : Endothelial Cell