Title: Nobiletin Attenuates Neurological Deficits, Blood-Brain Barrier Permeability, and Cerebral Edema in a Rat Model of Traumatic Brain Injury: Roles of IL-1 and IL-10

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

Received date: 2025/07/27

Revised date: 2026/05/25

Accepted date: 2026/08/01

Please cite this article as:
Alivirdiloo, V., Hajiabbasi, M., Siahposht Khachaki, A., Sadeghi, A., Bagheri-Mohammadi, S. (In Press). Nobiletin Attenuates Neurological Deficits, Blood-Brain Barrier Permeability, and Cerebral Edema in a Rat Model of Traumatic Brain Injury: Roles of IL-1 and IL-10. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.3050.3
DOI: http://dx.doi.org/10.32598/bcn.2026.3050.3

1. Abstract

Objective: To test whether nobiletin (NOB) improves neurological and motor function and mitigates secondary injury (edema, BBB disruption, histopathology) via modulation of IL-1β and IL-10 in a severe diffuse TBI rat model.

Methods: Adult male Wistar rats (n=56; 7 groups, 8-10 rats/group) underwent Marmarou weight-drop TBI or sham/controls: Intact, Sham, TBI, Vehicle/Saline, NOB 25 mg/kg, NOB 50 mg/kg, NOB 100 mg/kg. NOB or vehicle was administered intraperitoneally 30 min post-injury (single dose; [insert solvent and volume]). Neurological (VCS) and motor (beam walk, balance beam) scores were assessed on Days 0–3 by blinded evaluators. Brain water content and Evans blue extravasation were measured at 72 h. CSF IL-1β and IL-10 were quantified by ELISA. Histology (H&E, 5 µm) was evaluated blinded; [optional] semi-quantitative neuronal injury scores were assigned. Primary analysis for longitudinal behavior used two-way repeated-measures ANOVA; other endpoints used one-way ANOVA with Tukey's test; data are mean ± SEM.

Results: Compared with TBI/Vehicle, NOB 25 and 50 mg/kg improved VCS and motor performance across Days 1–3 (Group×Day interaction [insert F, p]). AUC analyses corroborated these effects. NOB 50 mg/kg most consistently reduced brain water content ([insert % reduction] vs TBI; p=[insert]) and Evans blue permeability ([insert % reduction]; p=[insert]). CSF IL-1β increased after TBI and was reduced by NOB 25 and 50 mg/kg ([insert values], p=[insert]); IL-10 decreased after TBI and was increased by NOB 25 and 50 mg/kg ([insert], p=[insert]). The 100 mg/kg dose showed limited or no benefit across endpoints. Histology revealed attenuated neuronal edema/pyknosis and vascular congestion with NOB 25–50 mg/kg; [if scored] injury scores were reduced vs TBI/Vehicle (p=[insert]).

Conclusion: A single post-injury dose of nobiletin at 25–50 mg/kg improved neurological outcomes and mitigated edema, BBB disruption, histopathology, and cytokine imbalance within 72 h after severe diffuse TBI, with 50 mg/kg showing the most consistent efficacy. The 100 mg/kg dose was not beneficial. Findings support anti-inflammatory and vasculoprotective actions of nobiletin in acute TBI.

Keywords: Nobiletin; Traumatic brain injury; Cytokines, IL-1β, IL-10

2. Introduction

Brain injury, particularly traumatic brain injury (TBI), ranks among the leading causes of death and disability in young people worldwide and is a common reason for hospital admissions [23]. According to the Centers for Disease Control and Prevention, approximately 1.7 million people in the United States suffer TBI each year, reflecting the rising global prevalence of trauma (1).

TBI triggers multiple cellular and molecular pathways that drive its pathogenic cascade. Brain damage from TBI occurs in two phases: the primary phase, which happens at the moment of impact and includes cerebral edema, intracranial hemorrhage, diffuse axonal injury, contusion, and blast injury; and the secondary phase, which follows [4, 21].

The primary injury leads to a secondary injury phase lasting days to weeks (or longer), characterized by biological processes aimed at restoring cellular homeostasis. However, these responses often exacerbate damage, promoting progressive neurodegeneration and cell death. Key features of secondary injury—which unfolds in a time-dependent manner—include blood-brain barrier disruption, oxidative stress, glutamate excitotoxicity, and neuroinflammation [17].

Nobiletin (NOB), or 3',4',5,6,7,8-hexamethoxyflavone, is a polymethoxylated flavone abundant in citrus peels, especially from oranges and lemons [20]. NOB has garnered attention for its pleiotropic effects, including anti-carcinogenic, anti-inflammatory, atheroprotective, anti-diabetic, and anti-obesity properties [7, 16, 11, 5].

Preclinical studies show NOB enhances memory in models of neurodegenerative diseases, such as Alzheimer's disease, and improves motor function and cognition in MPTP-induced parkinsonian mice. In Alzheimer's models, NOB reduces amyloid-β precursors in transgenic mice and those treated with NMDA receptor antagonists, while alleviating age-related cognitive decline, tau hyperphosphorylation, and oxidative stress. NOB also mitigates NMDA antagonist-induced memory deficits by activating intracellular kinase pathways, potentially via enhanced CREB gene transcription through PKA/ERK/MEK signaling [18, 12].

Cytokines—proteins released via paracrine and endocrine signaling—are pivotal for post-injury defense and repair. Their expression rises after trauma, with interleukin (IL) and TNF-α playing key roles in the neuroinflammatory response. IL-1 family members contribute prominently to secondary brain injury; notably, IL-1β peaks in brain tissue ~6 hours' post-injury and correlates with injury severity. Conversely, IL-10 inhibits pro-inflammatory cytokine production, suppresses cytokine receptor activity, and exerts neuroprotective effects in preclinical models [13, 22, 6, 8].

Given NOB's neuroprotective, anti-inflammatory, anti-apoptotic, and antioxidant effects—along with its ability to cross the blood-brain barrier, reduce ER stress, modulate autophagy, and inhibit excitotoxicity—this study examines whether NOB, administered via the Marmarou model, effectively mitigates diffuse brain injury. Furthermore, as NOB attenuates interleukins and neuroinflammation, we investigate whether the IL-1β/IL-10 pathway underlies its neuroprotective mechanism.

3. Materials and methods

Experimental animals and ethical approval

In this study, 56 male Wistar rats weighing 250–350 g were obtained from Mazandaran University of Medical Sciences, Iran. Rats were housed under standard controlled conditions (temperature: 21±2∘21±2∘C; humidity: 60–65%; 12/12 h light/dark cycle) and allowed to acclimate for 7 days before experiments began. All procedures were approved by the Ethics Committee of Mazandaran University of Medical Sciences (approval no. IR.MAZUMS.RES.1398.6054) and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. This study was funded by Mazandaran University of Medical Sciences, Ramsar Campus (grant no. 6054).

Experimental groups

In this study, the rats were randomly divided into seven groups (8-10 animals/each group) as follows: (1) control group (intact), (2) sham group, (3) traumatic brain injury group (TBI), (4) saline group (TBI + saline), (5) TBI + nobiletin-25 (NOB 25), (6) TBI + nobiletin-50 (NOB 50), (7) TBI + nobiletin-100 (NOB 100).

Experimental procedure

Wistar rats received various doses of nobiletin (25, 50, or 100 mg/kg) intraperitoneally 30 min after concussion induction via the Marmarou method. Behavioral assessments were conducted on day 0 (D0, immediately post-concussion), day 2 (D2), and day 3 (D3). Motor balance and coordination were evaluated using the beam walk and beam balance tests. Blood-brain barrier (BBB) permeability was assessed using Evans blue dye. After 72 h, cerebrospinal fluid (CSF) was collected and analyzed for levels of the pro-inflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10.

Assessment of behavioral and neurological outcomes

Vestibular motor performance and coordination were evaluated using beam balance and beam walk tests before and at 1, 2, and 3 days after traumatic brain injury (TBI). Neurological status was assessed using the Veterinary Coma Scale (VCS), as previously described (https://doi.org/10.1016/j.dscb.2022.100052).

Assessment the level of brain edema and blood-brain barrier integrity

Brain edema was quantified by the wet-dry weight technique. Fresh brain tissue was initially weighed (wet weight), then dried in an incubator at 60–70°C for 72 h before being weighed again (dry weight). The percentage of tissue water content was determined using the following formula: [(wet weight − dry weight)/wet weight] × 100. Blood–brain barrier (BBB) permeability was measured using the Evans blue extravasation assay, following the previously reported protocol (https://doi.org/10.1016/j.dscb.2022.100052).

Assessment of histopathological changes

At 24 h post-TBI, animals from each group were euthanized. Coronal brain sections (5 mm thick) were prepared (n=5 per group), fixed in 10% buffered formaldehyde for 24 h, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope by two independent pathologists in a blinded manner.

Level of inflammatory and anti-inflammatory cytokines in cerebrospinal fluid

Concentrations of IL-1β (pro-inflammatory) and IL-10 (anti-inflammatory) in CSF were measured 72 h post-TBI using ELISA kits according to the manufacturer's instructions.

Statistics

Results are expressed as mean ± SEM. Data were analyzed using GraphPad Prism software. Comparisons between groups were performed using one-way ANOVA followed by the Tukey-Kramer post hoc test. Differences were considered statistically significant at $P < 0.05$.

4. Results

Trauma-induced concussion caused cerebral edema, blood-brain barrier disruption, impaired neurological and balance scores, and dysregulated cytokine levels (decreased IL-10 and increased IL-1β) in our animal model. However, nobiletin (25 and 50 mg/kg) effectively reversed these effects compared to the vehicle-treated control group, with greater improvements at 50 mg/kg (Figures 4-1 to 4-8). After 72 hours, nobiletin administration significantly modulated inflammatory

as well as anti-inflammatory cytokines, decreasing IL-1β and increasing IL-10 (Figures 4-9 and 4-10).

Figure 4-1- Effects of NOB on neurological function in rats

Data show significant differences in neurological scores among groups at multiple time points post-TBI. At 30 min post-TBI, scores differed significantly between the intact and sham groups versus the TBI, saline, NOB 25, NOB 50, and NOB 100 groups ($P < 0.001$) (D0). According to the results, twenty-four hours after a concussion, there was a significant difference between the NOB 25, NOB 50, and NOB 100 groups with TBI and the Saline groups ($^{+}P < 0.05$, $^{+}P < 0.01$ and $^{+++}P < 0.001$). (D1). Also, 48 hours after a concussion, there were significant differences between the TBI and Saline groups that were determined to be $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, respectively (D2). Accordingly, for the Saline groups 72 hours after a concussion and the NOB 25, NOB 50, and NOB 100 groups with TBI, there was a significant difference ($^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$) (D3). The data are shown in mean and standard deviation. Six to eight rats are divided into each group (Fig. 1).

Figure 4-2- The area under the curve (AUC) of changes in the neurological scores of mice during 3 days

When comparing changes in animal neurological scores across four assessment sessions over three days (relative to pre-concussion baseline), the Intact group and also Sham the group exhibited the highest AUC. The AUC was lowest in the saline, TBI, and 100mg NOB groups. By giving NOB intraperitoneally at doses of 25 mg/kg and 50 mg/kg, the area under the curve was widened. In figure 2, comparatively to the Sham group and also Intact group, $^{***}P < 0.001$, $^{+}P < 0.01$ and $^{++}P < 0.05$ versus the TBI and Saline groups, respectively are shown. For the data, the average deviation from the norm is displayed. Six to eight rats are divided into each group (Fig. 2).

Figure 4-3-The level of brain water content

The data from statistical analysis revealed that the difference between the intact and sham groups is considerable ($^{***}P < 0.001$). Also the results indicated that the TBI and Saline groups differ significantly ($^{++}P < 0.01$ and $^{+++}P < 0.001$). In figure 3, the data is displayed as the average deviation from the standard. Six to eight rats are in each group (Fig. 3).

Figure 4- 4- The permeability of the blood-brain barrier in the studied groups

The data from figure 4 suggested that there is significant difference between treatment groups and intact and sham groups ($^{++}P < 0.01$), and ($^{+++}P < 0.001$) significant difference between treatment groups and TBI and Saline groups ($^{***}P < 0.001$). In figure 4, the information is presented as the average deviation from the standard. Each group contains 6 to 8 rats (Fig. 4).

Figure 4-5- vestibular-motor performance in the studied groups with the Beam-Walking method

Based on figure 5, there is ($^{***}P < 0.001$) significant difference between TBI, Saline, 25 NOB 25, NOB 50, and NOB 100 groups 30 minutes after concussion with intact and sham groups (D0). Also, there is ($^{++}P < 0.01$ and $^{+++}P < 0.001$) significant difference between NOB 25, NOB 50, and NOB 100 groups, TBI, and Saline groups 24 hours after concussion (D1). The data demonstrated that there was a significant difference ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$) between the NOB 25, NOB 50, and NOB 100 groups with TBI and the Saline groups 48 hours after trauma (D2).

Statistical analysis indicated that there is significant distinction ($^{\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$) for NOB 25, NOB 50, and NOB 100 groups with TBI and Saline groups 72 hours after concussion, respectively (D3). In figure 5, the information is presented as the average deviation from the standard. Each group contains 6 to 8 rats (Fig. 5).

Figure 4-6- Area under the beam-walking performance curve across the three-day assessment period for the experimental groups.

Statistical analysis revealed that the Intact and Sham groups had the smallest area under the curve. The groups with the most area under the curve were saline, TBI, and NOB 100mg. The area under the curve was reduced by administering NOB intraperitoneally at doses of 25 mg/kg and 50 mg/kg (Fig. 6). As shown in Figure 6, ***P < 0.001 vs. the Intact group and also Sham group; +$\boldsymbol{P}$ < 0.01 and ++$\boldsymbol{P}$ < 0.001 vs. the TBI and Saline groups.

Figure 4-7- vestibular-motor function in the studied groups with Beam-Balance method

The data demonstrated that 30 minutes after a concussion, there was a substantial difference between the intact and sham groups in the TBI, Saline, 25 NOB 25, NOB 50, and NOB 100 groups (D0). Also, 24 hours after a concussion, there was a significant difference between the NOB 25, NOB 50, and NOB 100 groups, TBI, and Saline groups ($^{++}P < 0.01$ and $^{+++}P < 0.001$). (D1). Furthermore, 48 hours after a concussion, there was a significant difference between the NOB 25,

NOB 50, and NOB 100 groups with TBI and the Saline groups ($^{\#}P < 0.05$, $^{\#\#} P < 0.01$ and $^{\#\#\#}P < 0.001$, respectively) (D2). Statistical analysis shown that significant differences were found in the NOB 25, NOB 50, and NOB 100 groups with TBI and Saline groups 72 hours after concussion, respectively ($^{\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$, respectively) (D3). In figure 7, the data is displayed as the average deviation from the norm. There are 6 to 8 rats in each group (Fig. 7).

Figure 4-8- The surface area under the Beam-Balance curve in the studied groups during 3 days of investigation

Rats' changes in vestibular-motor performance throughout the course of four experiments and three days (compared to before concussion induction) were most pronounced in the Intact and Sham groups. The saline, TBI, and NOB 100 mg groups had the lowest area under the curve. Intraperitoneal injections of NOB at doses of 25 mg/kg and 50 mg/kg increased the area under the curve. In figure 8, $^{***}P < 0.001$ compared the Sham and Intact groupings. Also, when compared to the TBI and Saline groups, $^{+}P < 0.05$ and $^{++}P < 0.01$ were used. Furthermore, the data is displayed as the average deviation from the norm. There are 6 to 8 rats in each group (Fig. 8).

Figure 4-9- The amount of cytokine IL-1β in cerebrospinal fluid

The data indicated that amount of the cytokine IL- β in cerebrospinal fluid was significantly increased by traumatic brain injury, and NOB 25 mg/kg and NOB 50 mg/kg could significantly decrease IL-1β. Statistical analysis demonstrated a significant difference between the Intact and Sham groups (***$\mathbf{P < 0.001}$**).** According to the figure 9, $^{+}P < 0.05$ and $^{++}P < 0.01$ show a significant difference between the TBI and Saline groups. In this figure, the information is presented as the average deviation from the standard. Each group contains 6 to 8 samples (Fig. 9).

Figure 4-10- The amount of cytokine IL-10 in cerebrospinal fluid

Based on results, after traumatic brain injury, the cytokine IL-10 in CSF fluid was dramatically decreased, whereas NOB 25 mg/kg and NOB 100 mg/kg may significantly increase IL-10.

The data revealed there was a significant difference between the intact and sham groups at $^{***}P < 0.001$, and between the TBI and saline groups at $^{+}P < 0.05$ and $^{++}P < 0.01$. In figure 10, the data are displayed as the average deviation from the norm. There are 6 to 8 samples in each group (Fig. 10).

Figure 4-11- Representative hematoxylin and eosin (H&E)-stained sections illustrating the effects of Nobiletin on histopathological changes at 72 h after TBI (400× magnification).

Figure (A) presents a representative histological section from the intact group. The brain tissue exhibited a normal histoarchitecture, characterized by well-preserved neurons with basophilic, euchromatic, oval-shaped cell bodies. Astrocytes also displayed normal morphology and distribution. Figure (B) illustrates the sham group, in which the overall brain architecture remained intact. Neurons exhibited large, centrally located vesicular nuclei, and glial cells showed normal histological features. In contrast, Figure (C) depicts the TBI group, where marked histopathological alterations were evident. Most neurons appeared edematous, irregular in shape, and contained hyperchromatic nuclei. Neuronal blebbing and tissue edema were frequently observed. In addition, astrocytes demonstrated pronounced vacuolization within the surrounding neuropil, accompanied by prominent pericellular halos. Enlarged endothelial cells and congested blood vessels were also evident.

Figure D showed the microscopic image in adult rats (Vehicle group). Degenerated neurons having wrinkled nuclei and intense staining, accompanied by perineural edema. Figure E showed the microscopic image in adult rats (TBI group) treated with NOB 25 mg/kg. Normal neurons with large central vesicular nuclei and glial cells are observed and all pathological changes were seen in the NOB 25 mg/kg group with reduced intensity. Figure F showed the microscopic image in adult rats (TBI group) treated with NOB 50 mg/kg. most neurons were normal in shape, with euchromatic nuclei and clear nucleoli. Similarly, endothelial cells, blood vessels, and astrocytes generally had normal morphology. Figure G showed the microscopic image in adult rats (TBI group) treated with NOB 100 mg/kg. The data revealed that the NOB 100 mg/kg group exhibited severe histopathological alterations comparable to those observed in the vehicle group. Degenerated neurons having wrinkled nuclei and intense staining, accompanied by perineural edema.

5. Discussion

Traumatic brain injury (TBI) triggers a cascade of inflammatory processes that exacerbate neuronal damage, cell death, dysfunction, and impaired recovery. This inflammation arises from astrocyte and microglial activation, along with infiltration of circulating leukocytes at the injury site. Activated glia release inflammatory mediators, including cytokines, chemokines, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Overproduction of these mediators induces neuronal toxicity, further amplifying glial activation and secondary neuronal injury [2, 3]. Inflammatory cytokines also activate receptor-dependent apoptotic pathways, engaging molecules such as caspase-8 or -10 [9, 10]. Thus, inhibiting glial activation and mediator production represents a promising therapeutic strategy for mitigating TBI-induced damage. However, anti-inflammatory drugs tested in clinical trials have shown limited success [14]. Nobiletin (NOB), a polymethoxylated flavonoid (3',4',5,6,7,8-hexamethoxyflavone) abundant in orange and lemon peels, exhibits anti-cancer, anti-inflammatory, anti-atherosclerotic, anti-diabetic, and anti-obesity effects [20]. It also demonstrates antidepressant and neuroprotective properties, as illustrated in Figure 1 [19]. Prior studies have not examined nobiletin's protective effects, optimal dosage, or administration timing in TBI. This study evaluated the therapeutic effects of nobiletin on severe traumatic brain injury (TBI) in mice by examining recovery of consciousness using the vestibulomotor consciousness score (VCS), an experimental analogue of the human Glasgow Coma Scale (GCS), motor performance through the beam-walking (BW) and beam-balancing (BB) tests, and two major pathological outcomes of TBI, namely brain edema and blood–brain barrier (BBB) disruption. BBB disruption promotes inflammatory cell infiltration, hemorrhage, and edema, risking substantial tissue damage and death. After 72 hours, we measured interleukin (IL)-1β and IL-10 levels in cerebrospinal fluid (CSF) to evaluate the pro- versus anti-inflammatory cytokine balance and nobiletin's anti-inflammatory role. Nobiletin mitigated severe TBI damage across all tested doses (25, 50, and 100 mg/kg). VCS improved significantly in nobiletin-treated groups from days 1–3 post-injury compared to TBI and saline controls; area under the curve (AUC) analysis confirmed efficacy at 25 and 50 mg/kg, with maximal effects at 50 mg/kg. Similarly, nobiletin at 25 and 50 mg/kg reduced brain water content and Evans blue extravasation, indicating decreased cerebral edema and BBB permeability (most pronounced at 50 mg/kg). Motor performance (BW and BB) improved at all doses over 3 days, with AUC significance at 25 and 50

mg/kg (optimal at 50 mg/kg). TBI elevated CSF IL-1β and suppressed IL-10, reflecting proinflammatory imbalance. Nobiletin at 25 and 50 mg/kg restored this balance by decreasing IL-1β and increasing IL-10 (most effectively at 50 mg/kg); the 100 mg/kg dose had no significant effect on either cytokine.

These findings align with prior evidence on polyphenols in neurodegenerative diseases. Polyphenols like carnosic acid, curcuminoids, luteolin, nobiletin, baicalin, and daidzein promote neuronal survival, growth, proliferation, and differentiation via key signaling pathways [15]. Orally administered nobiletin (200 mg/kg) enhanced memory in mice and supported PC12 cell survival in citrus extracts, linked to cAMP-responsive element-binding protein (CREB) activation during maze testing [13]. In cerebral ischemia models, nobiletin (25 mg/kg intraperitoneally) reduced NF-κB, TNF-α, IL-1β, and IL-6 levels, as well as reactive oxygen species [14]. Our study extends this by showing nobiletin reduces IL-1β and elevates IL-10 in TBI-induced CSF inflammation, alongside improvements in edema and neurological scores.

6. Conclusion

Nobiletin exerts therapeutic effects across multiple facets of the secondary phase of traumatic brain injury (TBI), including anti-inflammatory actions and vestibulomotor recovery. It confers significant neuroprotection by attenuating brain edema, histopathological damage, blood-brain barrier (BBB) permeability, and IL-1 levels while elevating IL-10, ultimately yielding improved neurological function.

Limitations

This study did not assess long-term outcomes, given TBI's complex pathophysiology, nor did it evaluate post-TBI memory or mood deficits. While IL-1β and IL-10 shifts in CSF confirmed nobiletin's anti-inflammatory effects, we did not identify mediating cell types or perform molecular/enzymatic analyses of its mechanisms. The 100 mg/kg dose lacked cytokine-modulating efficacy, possibly indicating a therapeutic window. TBI was induced via the Marmarou diffuse concussion model in rats, consistent with prior work but limiting generalizability.

7. **Funding**:

This study was funded by Mazandaran University of Medical Sciences, Ramsar campus

8. **Declaration of Competing Interest:**

The authors declare that they have no competing interests.

9. **Acknowledgments**:

The author acknowledges the faculty of medicine, Mazandaran University, Ramsar campus, Iran for supporting and funding through grant number 6054**.**

10. **Declaration of Generative AI and AI-assisted technologies in the writing process**

During the final English-language editing of this manuscript, the authors used artificial intelligence (AI) to improve the readability and clarity of selected sentences. Following the use of this tool, the authors carefully reviewed and revised the manuscript as necessary and accept full responsibility for the content of the published article.

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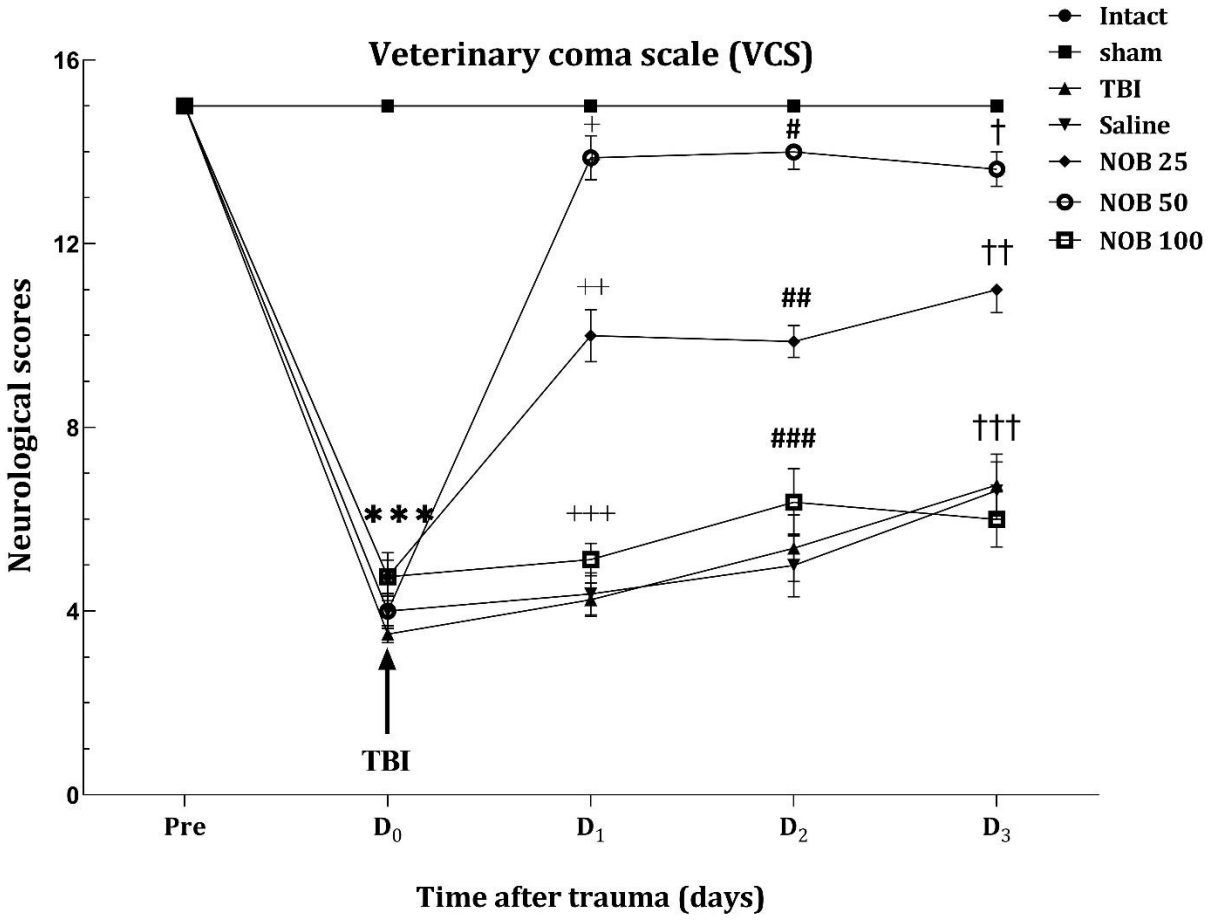
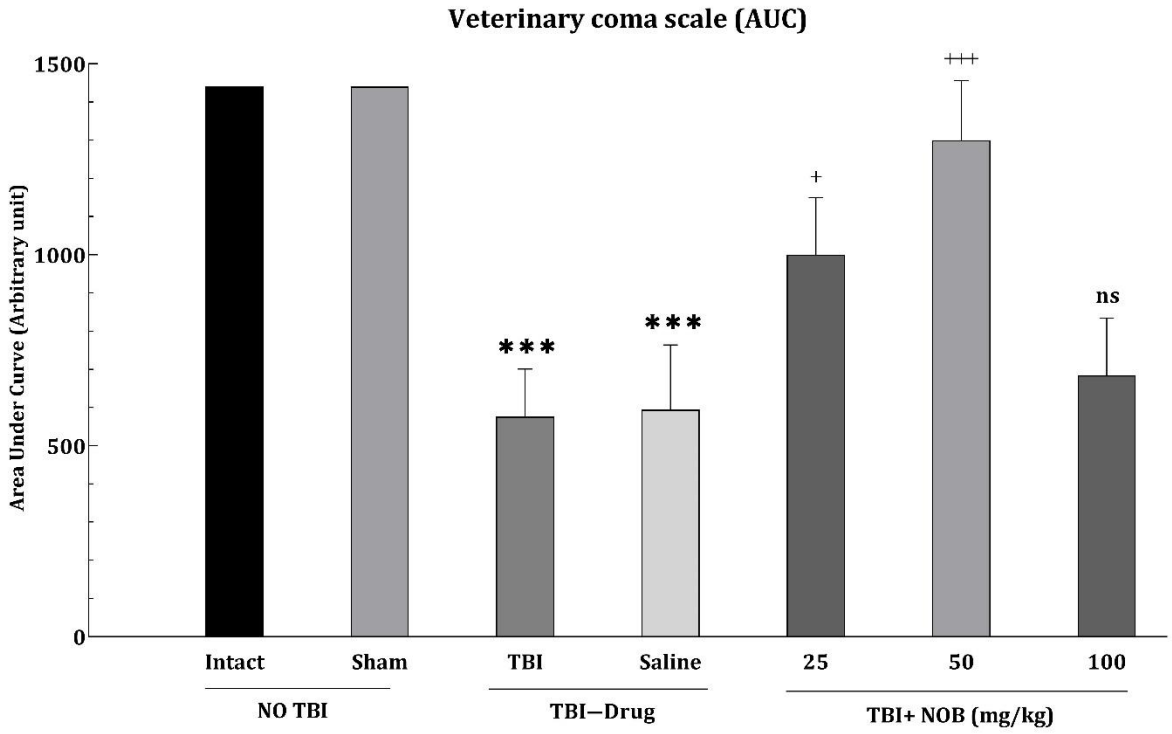
Figure 4-1- The effect of NOB on the neurological scores of rats



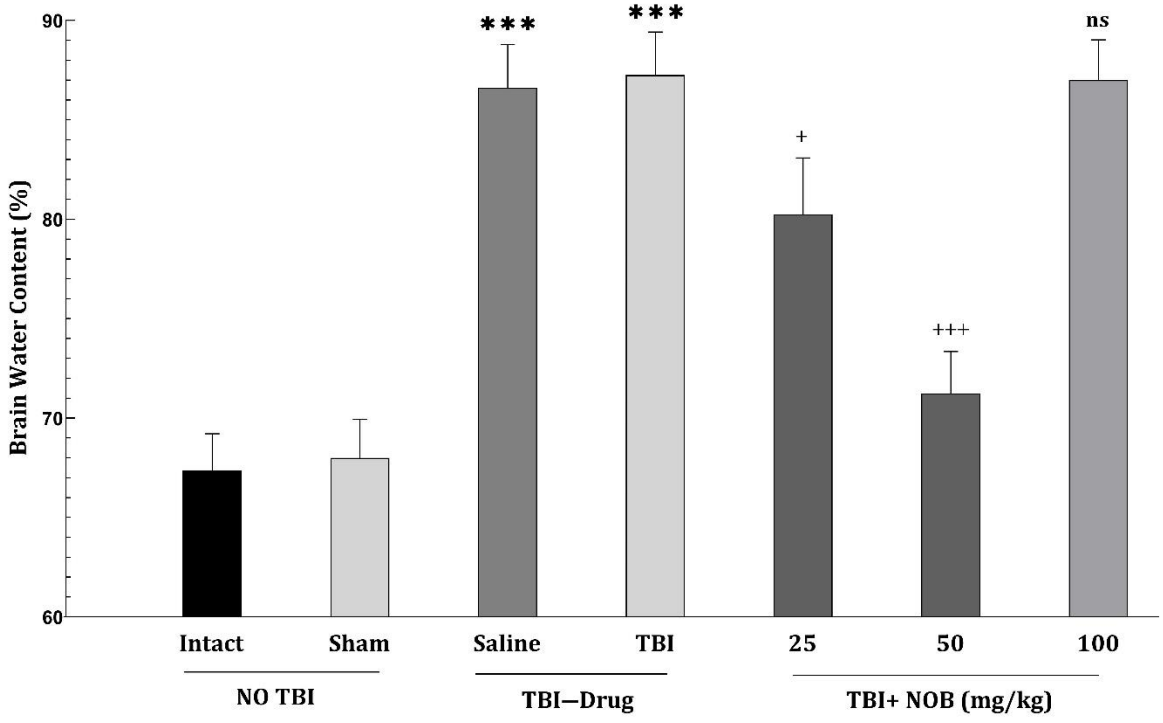
Data are presented as the mean ± SEM (n = 6–8 rats per group). ***P < 0.001 indicates a significant difference between the TBI, Saline, NOB 25, NOB 50, and NOB 100 groups and the Intact group and also Sham group on Day 0 (D0). +P < 0.05, ++P < 0.01, and +++P < 0.001 indicate significant differences between the TBI, Saline, NOB 25, NOB 50, and NOB 100 groups and the Intact group and also Sham group on Day 1 (D1). #P < 0.05, ##P < 0.01, and ###P < 0.001 indicate significant differences between the TBI, Saline, NOB 25, NOB 50, and NOB 100 groups and the Intact group and also Sham group on Day 2 (D2). †$\boldsymbol{P}$ < 0.05, ††$\boldsymbol{P}$ < 0.01, and †††$\boldsymbol{P}$ < 0.001 indicate significant differences between the TBI, Saline, NOB 25, NOB 50, and NOB 100 groups and the Intact group and also Sham group on Day 3 (D3).

Figure 4-2- The area under the curve of changes in the neurological scores of mice during 3 days



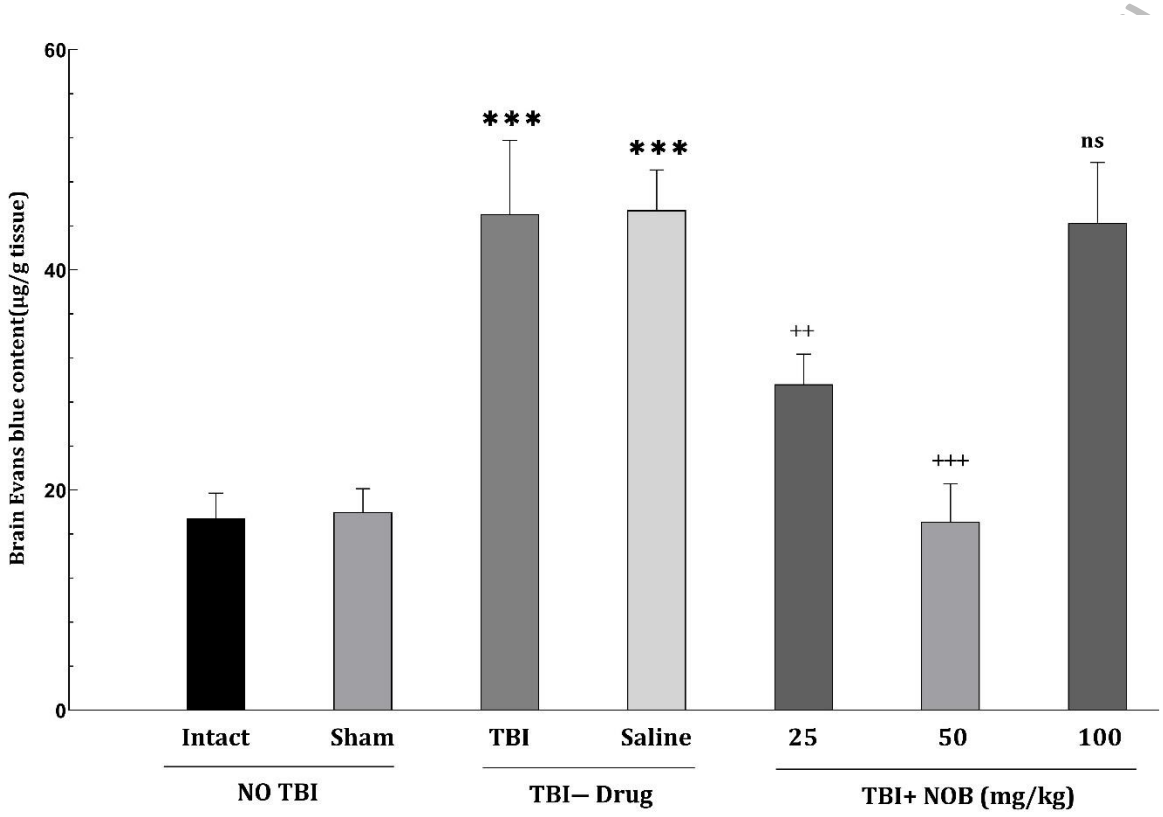
Area under the curve (AUC) for the vestibulomotor consciousness score (VCS) during the experimental period following traumatic brain injury (TBI) in male rats. The AUC was calculated using VCS measurements obtained before injury (Pre), on the day of injury (D0), and on post-injury Days 1 (D1), 2 (D2), and 3 (D3). The Intact group and also Sham group exhibited the highest AUC values, whereas the TBI, Saline, and NOB 100 mg/kg groups showed the lowest values. Intraperitoneal (i.p.) administration of nobiletin at doses of 25 and 50 mg/kg significantly increased the AUC compared with the TBI group and Saline group. Data are presented as the mean ± SEM. ***$P < 0.001$ vs. the Intact group and also Sham group; +$P < 0.05$ and +++$P < 0.001$ vs. the TBI and Saline groups.

Figure 4-3-The level of brain water content



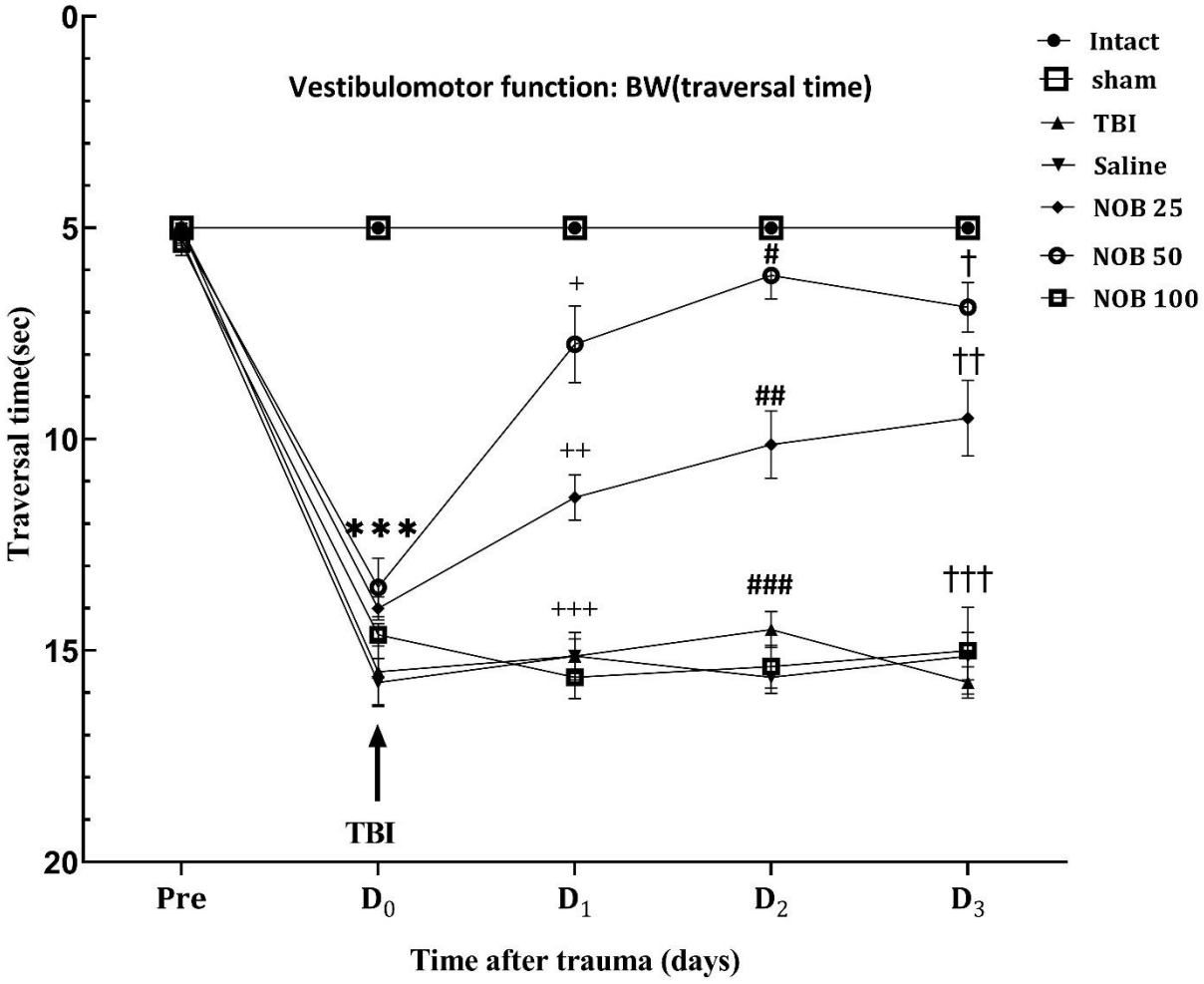
Data are presented as the mean ± SEM (n = 6–8 rats per group). ***P < 0.001 indicates a significant difference between the TBI and Saline groups and the Intact group and also Sham group. +P < 0.05 and +++P < 0.001 indicate significant differences between the NOB 25 mg/kg and NOB 50 mg/kg groups and the TBI and Saline groups.

Figure 4- 4- The permeability of the blood-brain barrier in the studied groups



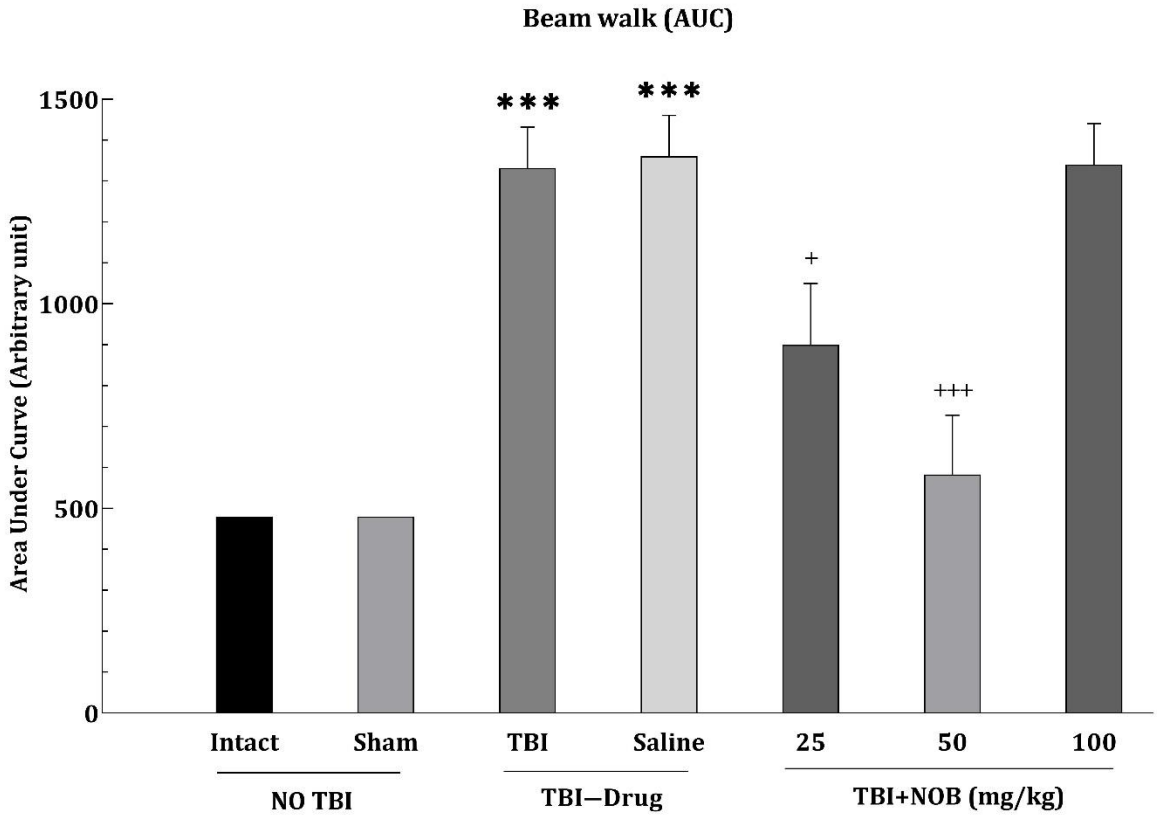
Data are presented as the mean ± SEM (n = 6–8 rats per group). ***$P < 0.001$ vs. the Intact group and also Sham group. ++$P < 0.01$ and +++$P < 0.001$ vs. the TBI and Saline groups.

Figure 4-5- vestibular-motor performance in the studied groups with the Beam Walk method



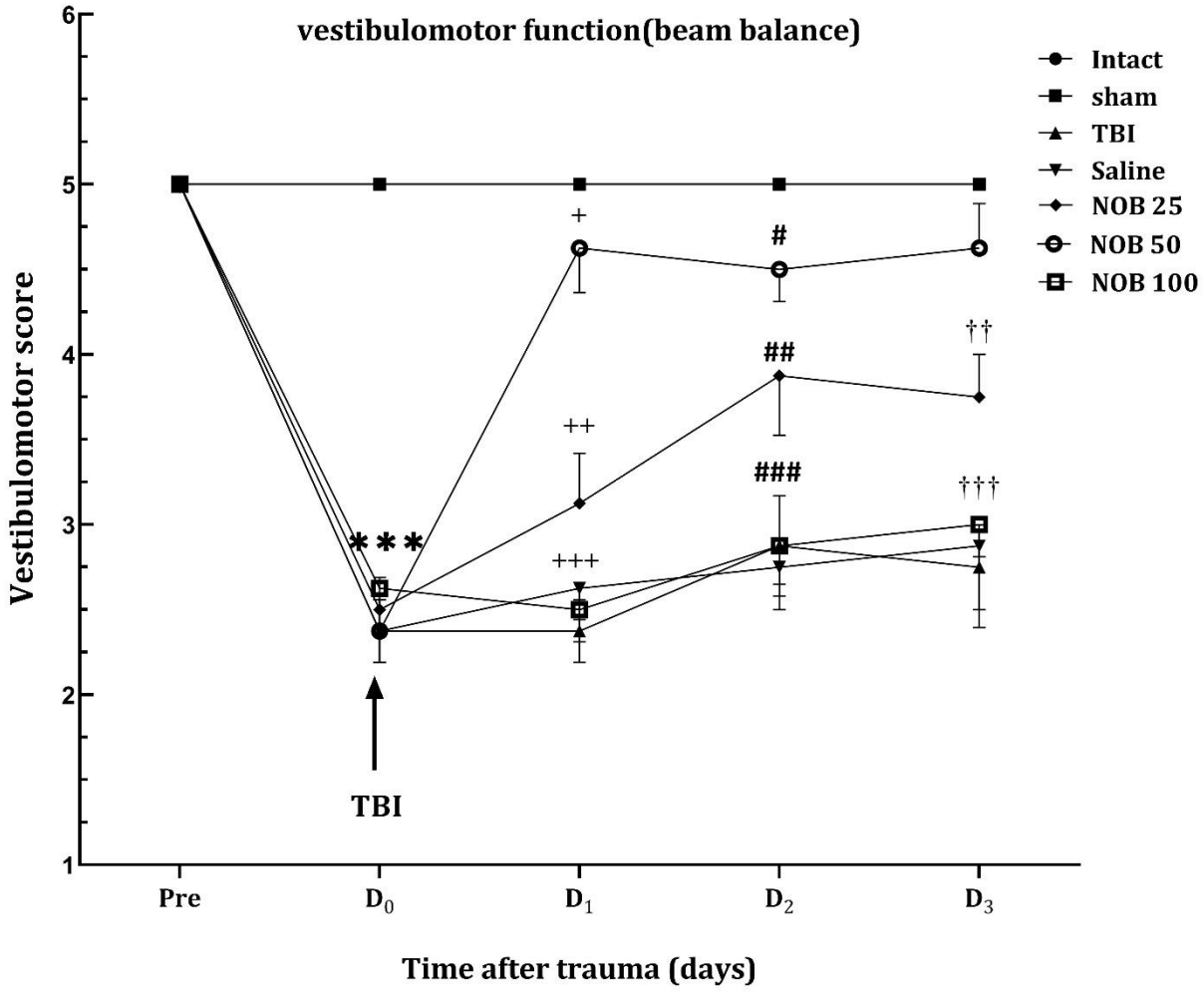
Data are presented as the mean ± SEM (n = 6–8 rats per group). ***P < 0.001 indicates a significant difference between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 0 (D0). +P < 0.05, ++P < 0.01, and +++P < 0.001 indicate significant differences between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 1 (D1). #P < 0.05, ##P < 0.01, and ###P < 0.001 indicate significant differences between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 2 (D2). †P < 0.05, ††P < 0.01, and †††P < 0.001 indicate significant differences between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 3 (D3).

Figure 4-6- The area under the beam walk curve in the studied groups during the 3 days of investigation



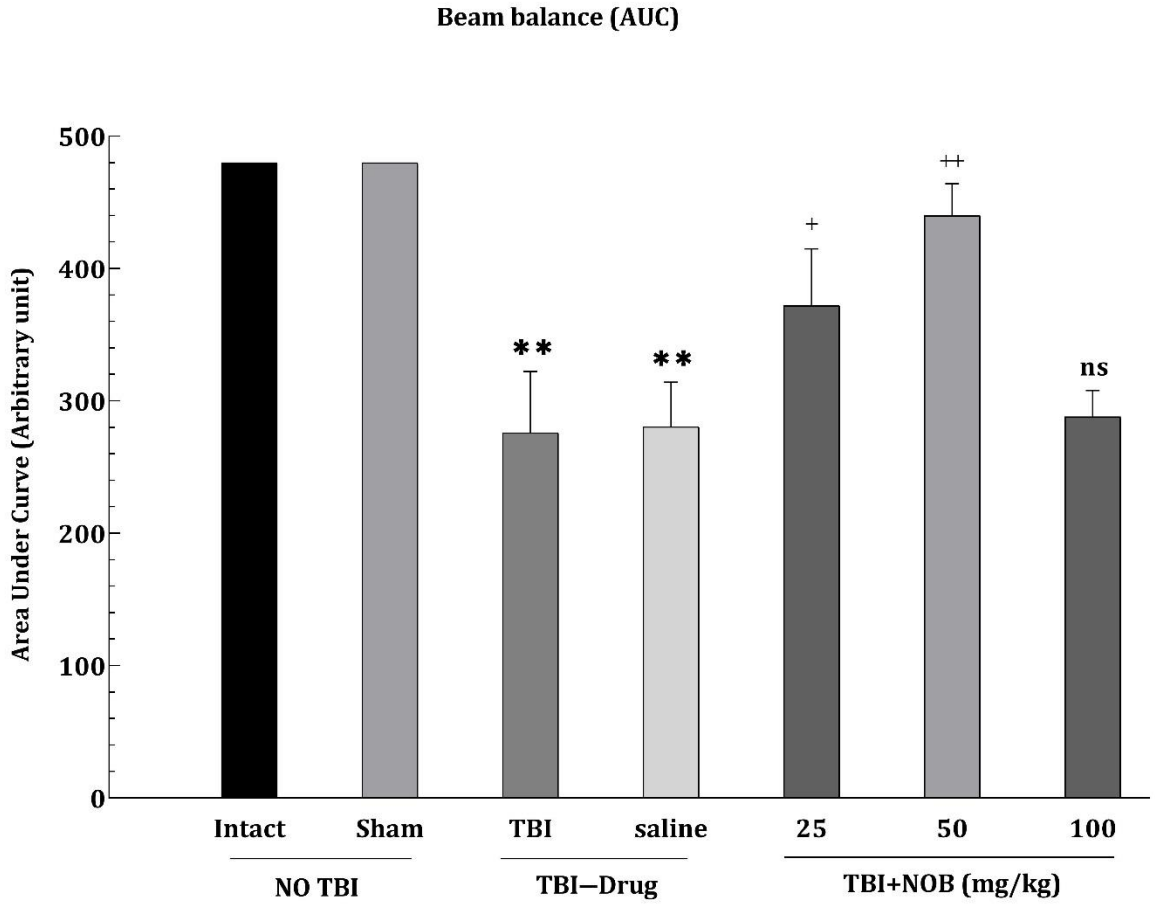
Area under the curve (AUC) for beam-walking performance during the experimental period following traumatic brain injury (TBI) in male rats. The AUC was calculated from beam-walking scores obtained before injury (Pre), on the day of injury (D0), and on post-injury Days 1 (D1), 2 (D2), and 3 (D3). The Intact group and also Sham group exhibited the lowest AUC values, whereas the TBI, Saline, and NOB 100 mg/kg groups showed the highest AUC values. Intraperitoneal (i.p.) administration of nobiletin at doses of 25 and 50 mg/kg significantly reduced the AUC compared with the TBI and Saline groups. Data are presented as the mean ± SEM. ***$P < 0.001$ vs. the Intact group and also Sham group; +$P < 0.05$ and +++$P < 0.001$ vs. the TBI and Saline groups.

Figure 4-7- vestibular-motor function in the studied groups with Beam Balance method



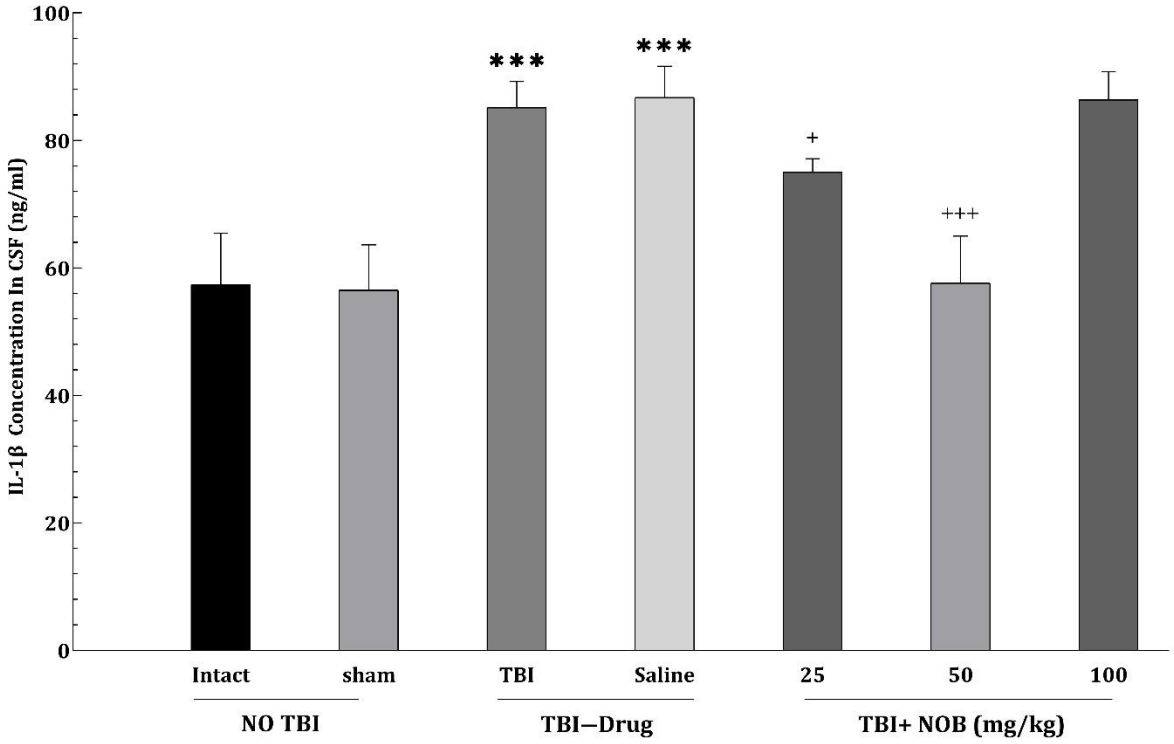
Data are presented as the mean ± SEM (n = 6–8 rats per group). ***P < 0.001 indicates a significant difference between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 0 (D0). +P < 0.05, ++P < 0.01, and +++P < 0.001 indicate significant differences between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 1 (D1). #P < 0.05, ##P < 0.01, and ###P < 0.001 indicate significant differences between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 2 (D2). ††P < 0.01 and †††P < 0.001 indicate significant differences between the TBI, Saline, NOB 25 mg/kg, NOB 50 mg/kg, and NOB 100 mg/kg groups and the Intact group and also Sham group on Day 3 (D3).

Figure 4-8- The surface area under the Beam Balance curve in the studied groups during 3 days of investigation



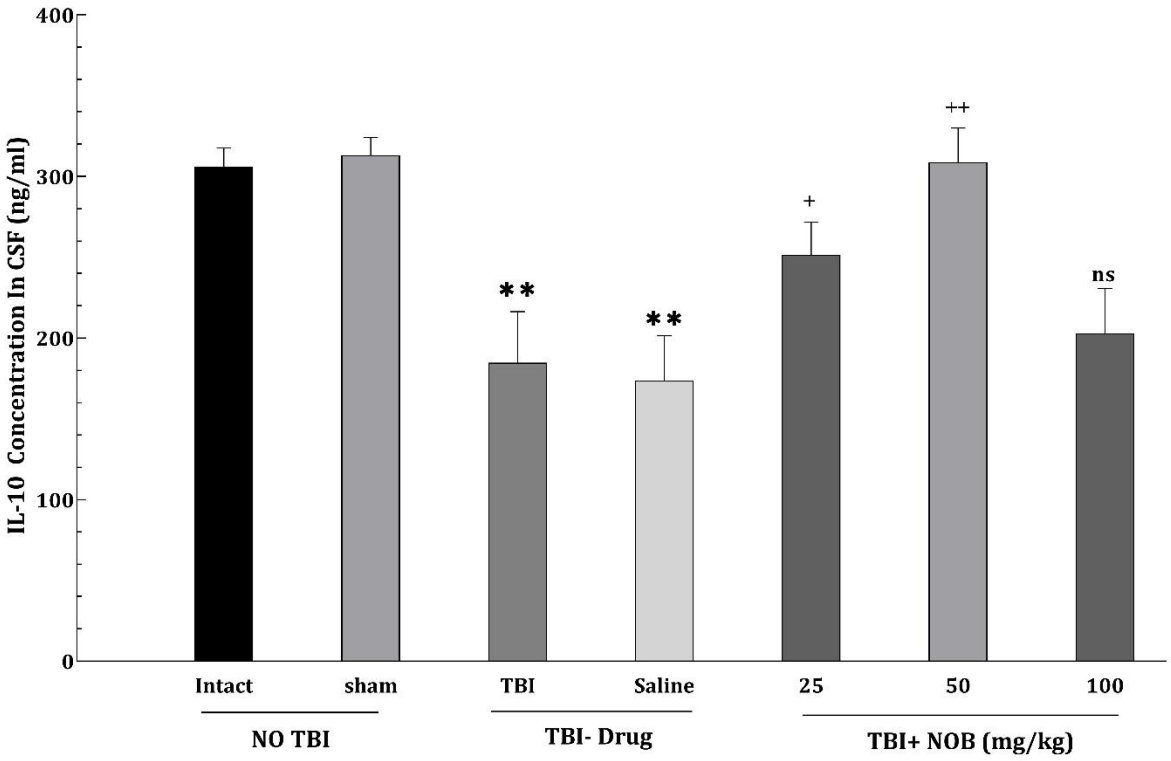
Area under the curve (AUC) for beam-balancing performance during the experimental period following traumatic brain injury (TBI) in male rats. The AUC was calculated from beam-balancing scores obtained before injury (Pre), on the day of injury (D0), and on post-injury Days 1 (D1), 2 (D2), and 3 (D3). The Intact group and also Sham group exhibited the highest AUC values, whereas the TBI, Saline, and NOB 100 mg/kg groups showed the lowest AUC values. Intraperitoneal (i.p.) administration of nobiletin at doses of 25 and 50 mg/kg significantly increased the AUC compared with the TBI and Saline groups. Data are presented as the mean ± SEM. ***P* < 0.01 vs. the Intact group and also Sham group; +*P* < 0.05 and ++*P* < 0.01 vs. the TBI and Saline groups.

Figure 4-9- The amount of cytokine IL-1β in cerebrospinal fluid



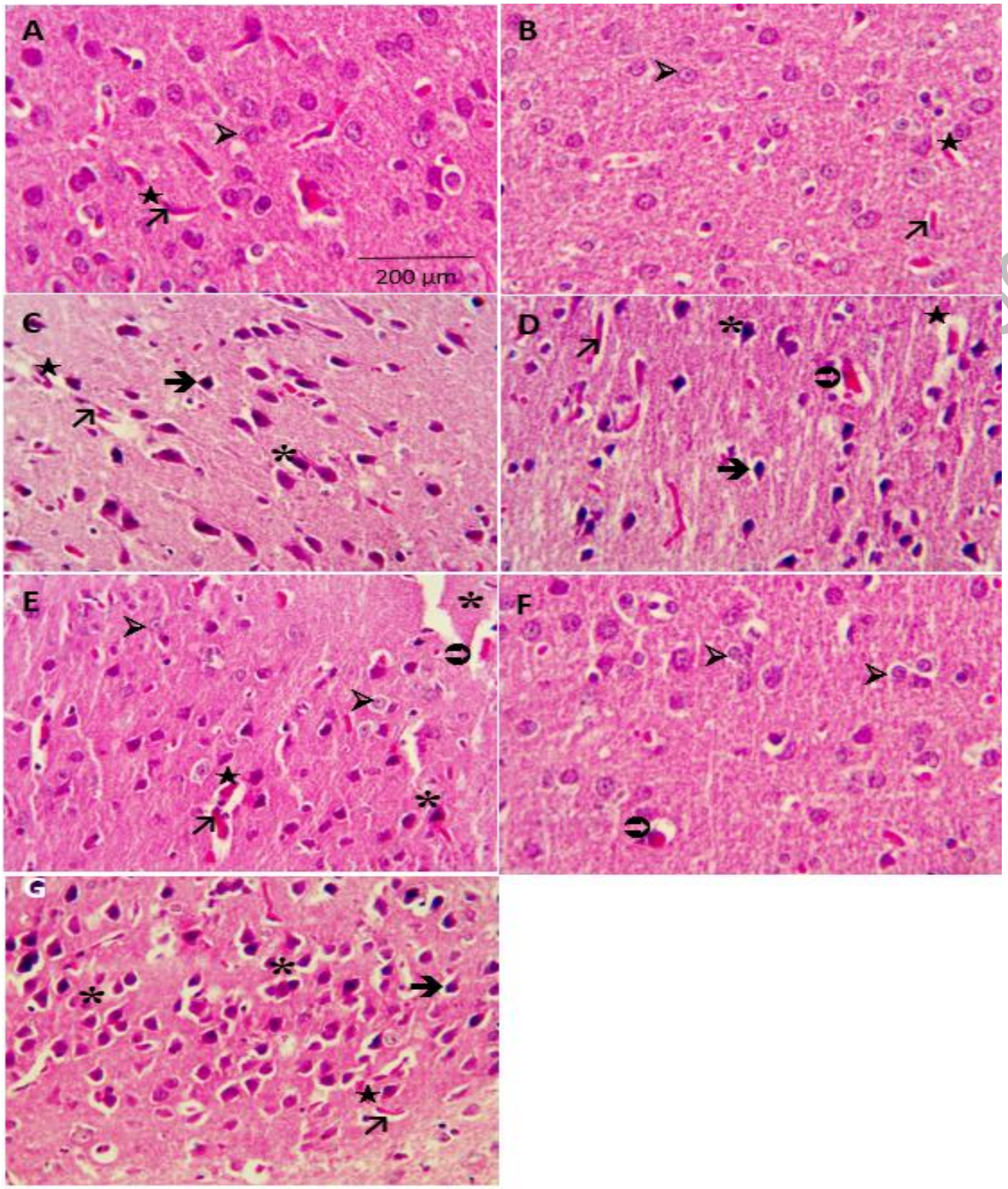
Data are presented as the mean ± SEM (n = 6–8 rats per group). ****P* < 0.001 vs. the Intact group and also Sham group. +*P* < 0.05 and +++*P* < 0.001 indicate significant differences between the NOB 25 mg/kg and NOB 50 mg/kg groups and the TBI and Saline groups.

Figure 4-10- The amount of cytokine IL-10 in cerebrospinal fluid



Data are presented as the mean ± SEM (n = 6–8 rats per group). ***P* < 0.01 vs. the Intact group and also Sham group. +*P* < 0.05 and ++*P* < 0.01 indicate significant differences between the NOB 25 mg/kg and NOB 50 mg/kg groups and the TBI and Saline groups.

Figure 4-11- The effects of Nobiletin on histopathology changes in 72 h post-TBI with 400 X magnification



A) intact B) sham, C) TBI group, D) Saline, E) NOB 25 mg/kg, F) NOB 50 mg/kg, G) NOB 100 mg/kg in male animal (brain).

➢ : Normal neuron, ⁎: Degenerated neuron, ➔: Edematous neuron, ➲:Swollen Astrocyte, ★: Blood Vessel, ↗: Endothelial Cell