International Journal of Health Sciences
Available online at www.sciencescholar.us
E-ISSN: 2550-696X
Int. J. of Health Sci.
DOI: 10.53730

How to Cite:
Elbaradei, H. F., Ismail, G., Rizk, F. H. A. E., & Musallem, A. H. H. (2026). Effect of berberine on thioacetamide-induced hepatic encephalopathy: Possible modulation of gut microbiota-liver-brain axis in rats. *International Journal of Health Sciences*, *10*(S1), 278–294.
https://doi.org/10.53730/ijhs.v10nS1.16003

Effect of berberine on thioacetamide-induced hepatic encephalopathy: Possible modulation of gut microbiota-liver-brain axis in rats

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***Abstract*---Background:** Hepatic encephalopathy (HE) is a severe neuropsychiatric complication of liver disease associated with hyperammonemia, gut microbiota dysbiosis, inflammation, and oxidative stress along the gut–liver–brain axis. This study investigated the protective effects of berberine (BBR) against thioacetamide (TAA)-induced HE in rats, with particular emphasis on its modulation of the gut–liver–brain axis. **Methods:** Forty male Wistar rats were divided into four groups: control, BBR control, TAA-induced HE, and BBR-treated HE. HE was induced with TAA for 2 weeks, while BBR was

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Submitted: 09 April 2026, Revised: 18 June 2026, Accepted: 27 July 2026

administered orally throughout the same period. Cognitive function, biochemical markers, and histopathological changes in the liver, brain, and colon were evaluated. **Results:** TAA significantly increased ALT, AST, ammonia, LPS, TLR-4, NF-κB, and MDA, while decreasing SCFAs, glutamic acid, GSH, and ZO-1 levels ($p<0.05$). BBR significantly reversed these changes, improved cognitive function, and restored histopathological architecture in liver, brain, and colon. However, all parameters in the BBR-treated HE G continued to vary significantly from the control G ($p < 0.05$), reflecting partial recovery. **Conclusions:** BBR exerts multi-targeted protective effects against TAA-induced HE via regulation of the gut-liver-brain axis, suggesting its utility as a therapeutic agent for HE. Further investigations are warranted to explore its full therapeutic potential.

***Keywords*---**Hepatic Encephalopathy, Berberine, Thioacetamide, Gut-Liver-Brain Axis, Neuroinflammation, Oxidative Stress.

1. Introduction

Hepatic encephalopathy (HE) is a severe neuropsychiatric disorder resulting from acute or chronic liver injury and is known by impaired central nervous system function. It frequently develops when the liver is unable to eliminate toxins from the circulation and is accompanied by cognitive deficits, motor impairment, reduced responsiveness, and somnolence (Acharya & Bajaj, 2018). According to epidemiological studies, the 5-year cumulative cirrhosis incidence of HE has been reported to range from 5% to 25% (Elsaid & Rustgi, 2020). The researchers concluded that hyperammonemia is a key contributor to HE (Mondal & Trigun, 2014). The intestinal microbiota is the principal contributor to endogenous ammonia production. HE is related with microbial dysbiosis and compromised intestinal barrier (IB) function, leading to enhanced intestinal permeability and increased passage of ammonia and inflammatory molecules into the circulation, where they contribute to brain injury via the gut–liver–brain axis (Mondal & Trigun, 2014).

Furthermore, hyperammonemia and inflammatory processes enhance the production of reactive oxygen species (ROS), thereby inducing oxidative stress (OS), compromising endogenous antioxidant defenses, and diminishing the viability of neuronal and hepatic cells in HE (Saleh, Mansour, & Fayez, 2021). Therefore, dysregulation of the gut–liver axis is a key contributor to the pathogenesis of HE. In addition, inflammatory responses and OS triggered by gut-derived toxins, particularly ammonia and lipopolysaccharide (LPS), further aggravate disease progression (Zhou et al., 2023).

Thioacetamide (TAA) is a frequently used hepatotoxin to induce HE models by causing OS in the liver, brain (Ferah Okkay et al., 2022)and disturbance of gut microbiota and IB, which can result in the liberation of inflammatory cytokines (CKs) (Zhou et al., 2023). Moreover, after TAA injection, the resulting neurobehavioral abnormalities resemble HE in humans (L. Yang et al., 2020).

BBR is an Isoquinoline alkaloid that is derived from the Coptis chinensis plant. It has been used clinically for many years and has a number of pharmacological impacts, as antibacterial, antioxidant and anti-inflammatory (Z. Zhu et al., 2020). Previous investigation proved that therapeutic efficacy of berberine (BBR) in modulating inflammatory responses appears to be mediated via its impacts on the intestinal microbiota (Fang et al., 2021).

However, to our knowledge, the impact of BBR on HE and gut microbiota-liver-brain axis have not been investigated. Therefore, our study designed to assess the potential mitigating impact of BBR on HE and gut microbiota-liver-brain axis components. Accordingly, the aim of our work was to examine the impact of BBR on thioacetamide-induced HE and investigate possible involvement of gut microbiota- liver- brain axis in this effect.

2. Materials and Methods:

2.1. Ethical Approval:

All experimental procedures were executed grounded on the standards for the care and use of laboratory animals and authorized by the Institutional Animal Ethics Committee of Faculty of Medicine, Tanta University under code no 36264MD88/5/23. All reasonable efforts were made to minimize animal suffering and to use the minimum number of animals required to achieve the study objectives.

2.2. Animals:

Adult male Wistar rats weighing 200–250 g were procured from the Laboratory Animal Center, Tanta University, Egypt. Throughout the study, the animals were kept in standard polypropylene cages under standardized laboratory conditions, as an ambient temperature of 22 ± 2°C, 50–55% relative humidity, and a 12-h light/dark cycle with illumination beginning at 07:00. Standard pellet chow and tap water were available ad libitum. A 2-week acclimatization period was allowed before the initiation of the experimental procedures.

2.3. Experimental Design and Groups (Gs):

Following acclimatization, forty rats were randomly placed into four Gs (n=10 per G): G I (Control): Had distilled water via oral gavage (50 mg/kg/day) for 2 weeks and normal saline intraperitoneally (ip) once every 48 hours (three times per week) as vehicle control. G II (BBR Control): Had BBR chloride (50 mg/kg/day) formulated as a suspension in distilled water and given once daily by oral gavage for two consecutive weeks. G III (TAA-HE): Had TAA prepared in sterile 0.9% saline and injected ip at a dose of 200 mg/kg three times weekly over a 2-week period to induce HE. G IV (BBR+HE): Had TAA as in G III plus BBR chloride (50 mg/kg/day) as in G II for 2 weeks. BBR treatment was commenced concurrently with the initial TAA administration and was administered once daily throughout the induction period.

2.4. Drugs and Chemicals:

TAA and BBR chloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). TAA was freshly prepared in sterile 0.9% saline before each injection. BBR chloride was dissolved in distilled water immediately prior to oral delivery, with

the volume adjusted according to individual animal body weight. All ELISA kits were obtained from commercial suppliers (Spectrum, Assay Genie, MyBioSource, Elabscience, Biodiagnostics, Cloud-Clone Corp).

2.5. Behavioral Assessment (Passive Avoidance Test):

Passive avoidance testing was performed on day 13 of the experiment to evaluate memory function. The passive avoidance apparatus consisted of two interconnected compartments: a lighted chamber constructed of white Plexiglas and a dark chamber made of black Plexiglas. The dark compartment was equipped with a grid floor to deliver mild electric foot shocks, while a sliding door allowed access among the two compartments.

2.5.1. Training Phase:

Each rat underwent three training trials with 30-minute intervals. Rats were kept individually in the lighted compartment; upon entering the dark compartment, an electric foot shock (50 Hz, 2 seconds duration, 0.8 mA intensity) was applied immediately.

2.5.2. Retention Phase:

24 h after the training session, memory retention was assessed in the absence of electric shock. For the retention test, each rat was introduced into the lighted compartment, and both the latency to enter the dark compartment (step-through latency) and the duration of stay in the dark compartment were measured. A shorter step-through latency was interpreted as impaired memory retention, whereas a longer latency indicated preserved memory performance.

2.6. Euthanasia and Sample Collection:

At the conclusion of the experimental period, the rats were deeply anesthetized via ip injection of sodium phenobarbital (60 mg/kg). Adequate anesthesia was verified by loss of righting and pedal reflexes. Animals were then sacrificed by cervical dislocation, consistent with approved euthanasia guidelines. Blood samples were collected by intracardiac puncture without anticoagulant in clean plastic tubes, allowed to clot before being centrifuged at 3,000 rpm for 15 min. Following separation, the sera were aliquoted into sterile tubes and preserved at −80°C until analysis. The liver, brain, and colon were carefully harvested and gently rinsed with chilled phosphate-buffered saline (PBS; pH 7.4). Each tissue specimen was subsequently sectioned into two portions. One portion was snap-frozen and preserved at −80°C for subsequent biochemical examination, whereas the remaining portion was fixed in 10% neutral-buffered formalin for subsequent histopathological examination. For biochemical assays, the frozen tissue samples were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.5) using a tissue homogenizer while maintaining cold conditions. The homogenates were then centrifuged at 3,000 rpm for 15 min, after which the resulting supernatants were gathered and preserved at −80°C until further examination.

2.7. Biochemical Analyses:

Liver Function Tests: Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined via commercial colorimetric kits (Spectrum, Egypt) grounded on the Reitman-Frankel method. Absorbance was determined at 546 nm, and enzyme activities were determined from standard conversion tables provided by the manufacturer.

Serum and Brain Ammonia: Ammonia concentrations were quantified using a colorimetric assay kit (Assay Genie) based on enzymatic conversion of ammonia to glutamate-by-glutamate dehydrogenase, with NADH oxidation monitored at 340 nm. Samples were prepared as per manufacturer instructions, and concentrations were interpolated from standard curves (0–1000 μM). Serum LPS: LPS levels were determined via a sandwich ELISA kit (MyBioSource). Absorbance was recorded at 450 nm, and concentrations were determined from standard curves (15.6–1000 ng/mL). Serum Short-Chain Fatty Acids (SCFAs): SCFAs were determined via a competitive ELISA kit (MyBioSource). Color intensity was indirectly related to SCFA concentration, determined at 450 nm. Brain Glutamic Acid: Glutamic acid in brain tissue was measured colorimetrically (Elabscience) using glutamate dehydrogenase-catalyzed reaction with NAD^+, monitoring NADH formation at 340 nm. Malondialdehyde (MDA): Tissue MDA levels in liver and brain were examined by thiobarbituric acid reactivity (Biodiagnostics), with absorbance determined at 534 nm. MDA concentrations were calculated using a standard curve (10 nmol/mL). Reduced Glutathione (GSH): GSH levels in liver and brain were determined by reaction with DTNB (Biodiagnostics), producing a yellow chromogen measured at 405 nm.

Nuclear Factor Kappa B (NF-κB): Tissue NF-κB protein levels were determined via a sandwich ELISA kit (MyBioSource), with absorbance read at 450 nm. Concentrations were interpolated from standard curves (0.312–20 ng/mL). Toll-Like Receptor 4 (TLR-4): TLR-4 levels in liver and brain were determined via an ELISA kit (Cloud-Clone Corp), with absorbance read at 450 nm. Zonula Occludens-1 (ZO-1): Intestinal and brain ZO-1 levels were determined via a sandwich ELISA kit (MyBioSource), with absorbance read at 450 nm. Concentrations were determined from standard curves (31.25–2000 pg/mL). All biochemical assays were executed in duplicate based on manufacturer protocols, with acceptable standard curve linearity ($R^2 > 0.99$). Samples exceeding the highest standard were appropriately diluted, reassayed, and concentrations multiplied by the dilution factor.

2.8. Histopathological Examination:

Formalin-fixed tissues were executed via graded ethanol series, the tissues were cleared in xylene and embedded in paraffin wax. Paraffin-embedded tissues were sectioned at a thickness of 4–6 μm using a microtome, mounted onto glass slides, and stained with hematoxylin and eosin (H&E). Histopathological examination of the brain, liver, and colon sections was performed under a light microscope by a pathologist who was blinded to the experimental G allocation.

2.9. Statistical Analysis:

It was executed via SPSS version 28 (Chicago, IL, USA). Data normality was examined via the Shapiro-Wilk test. Normally distributed data were shown as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was utilized for comparisons among Gs. Statistical significance was set at $p < 0.05$ with a 95% confidence interval.

3. Results

As shown in **Table 1**, no significant differences were documented among control G and BRB- treated control G as regards time in the dark and latency period during behavioural test. Time in the dark was significantly longer, and latency period was significantly lower among TAA- HE G versus control G and BRB-treated control G. Time in the dark was significantly shorter, and latency period was significantly longer among BBR- treated HE G versus TAA- HE G. Time in the dark was significantly longer, and latency period was significantly lower among BBR- treated HE G versus control G and BRB- treated control G.

As shown in **Table 2**, no significant differences were documented among the control G and BBR-treated control G ALT, AST, serum ammonia, serum LPS, serum SCFAs, brain ammonia and brain glutamic acid. Serum ALT, AST, ammonia, LPS, and brain ammonia levels were significantly higher, while serum SCFAs and brain glutamic acid levels were significantly lower in the TAA-HE G versus both the control G and BBR-treated control G. Serum ALT, AST, ammonia, LPS, and brain ammonia levels were significantly reduced, while serum SCFAs and brain glutamic acid levels were significantly raised in the BBR-treated HE G versus the TAA-HE G. However, serum ALT, AST, ammonia, LPS, brain ammonia, serum SCFAs, and brain glutamic acid levels in the BBR-treated HE G remained significantly different versus both the control G and BBR-treated control G, reflecting partial but incomplete reestablishment of biochemical parameters following BBR treatment.

As shown in **Table 3**, no significant differences were reported among the control G and BBR-treated control G regarding any hepatic or brain inflammatory and OS markers. Hepatic and brain TLR-4, NFκB, and MDA levels were significantly higher, while hepatic and brain GSH levels were significantly reduced in the TAA-HE G versus both the control G and BBR-treated control G. Hepatic and brain TLR-4, NFκB, and MDA levels were significantly reduced, while hepatic and brain GSH levels were significantly elevated in the BBR-treated HE G versus the TAA-HE G. Nevertheless, hepatic and brain TLR-4, NFκB, MDA, and GSH levels in the BBR-treated HE G remained significantly different versus both the control G and BBR-treated control G, reflecting partial but incomplete amelioration of hepatic and neuroinflammation and OS following BBR treatment. No significant differences were reported among the control G and BBR-treated control G regarding brain and intestinal ZO-1 levels. Both brain and intestinal ZO-1 levels were significantly reduced in the TAA-HE G versus both the control G and BBR-treated control G. Brain and intestinal ZO-1 levels were significantly elevated in the BBR-treated HE G versus the TAA-HE G. However, both brain and intestinal ZO-1 levels in the BBR-treated HE G remained significantly reduced compared to both the control G and BBR-treated control G, reflecting partial but incomplete reestablishment of blood-brain barrier (BBB) and IB integrity following BBR treatment.

Brain tissue histopathological examination revealed normal findings of both cerebral cortex and all regions of hippocampus in control and BBR- treated control Gs **(Figures 1AI, 1AII, 1AIII, 1AIV).** In TAA- HE G; there were notable histopathological alteration in cerebral cortex of both sides and all regions of

hippocampus. The cerebral cortex showed gliosis with numerous degenerated neurons and congested blood vessels **(Figure 1AV).** The different regions of hippocampus revealed numerous dark degenerating neurons in different regions **(Figure 1AVI).** Treatment with BBR was able to reverse these findings as both cerebral cortex and all regions of hippocampus showed marked improvement and marked reduction of the degenerated neurons in BBR- treated HE G **(Figures 1AVII, 1AVIII).**

Histopathological examination of liver samples of the studied Gs was done using hematoxylin and eosin staining. Control G and BBR- treated control G had normal hepatic parenchyma, normal architecture of hepatocytes and portal areas **(Figures 1BI, 1BII).** Induction of hepatotoxicity by TAA lead to significant changes in hepatic parenchyma and cellular components and structure as liver histopathological examination of TAA- HE G showed disturbed hepatic lobular architecture with marked fibrosis and ballooning degeneration of hepatocytes. Also, dilated congested central vein, dilated congested sinusoids were observed with bile ductular proliferation. Marked infiltration with inflammatory cells was noticed **(Figure 1BIII).** Treatment with BBR resulted in significant improvement of liver histopathological findings as liver histopathology of BBR- treated HE G showed limited portal fibroplasia and mild vacuolation of hepatic parenchyma suggesting regeneration **(Figure 1BIV).**

Histopathological examination of colonic tissues revealed clear differences among the experimental Gs. The control G demonstrated normal colonic architecture, with uniformly arranged, regular mucosal glands lined by goblet cells and no evidence of inflammatory infiltration, confirming intact mucosal integrity **(Figure 1CI).** Similarly, the BBR-treated control G demonstrated preserved colonic structure with regular glandular organization and abundant goblet cells, and no detectable inflammation, reflecting that BBR treatment alone did not induce any histopathological changes **(Figure 1CII).** In contrast, the TAA-HE encephalopathy G exhibited marked pathological alterations, including dense inflammatory cell infiltrates that disrupted the normal glandular arrangement of the colonic mucosa, signifying severe inflammatory injury and mucosal barrier compromise secondary to TAA toxicity **(Figure 1CIII).** Conversely, the BBR-treated HE G showed a notable attenuation of these lesions, with reestablishment of mucosal glandular structure and only mild inflammatory infiltration, reflecting the anti-inflammatory and mucosal protective impacts of BBR on colonic tissue in HE **(Figure 1CIV).**

4. Discussion

HE is a serious neuropsychiatric adverse event of liver disorder, affecting 30–40% of cirrhosis patients and leading to poor outcomes, hospitalization, and impaired quality of life (Ohikere & Wong, 2024). Ammonia plays a central role in its pathogenesis, with elevated levels causing astrocyte swelling and glutamine accumulation, while IB disruption enhances endotoxin translocation, exacerbating inflammation (Luo et al., 2023). TAA is frequently used to induce experimental HE, mimicking human pathology through OS and hepatocellular necrosis (Ezhilarasan, 2023b; Nassar, Abdalfattah, Hassan, & Nasif, 2021). Berberine, a natural alkaloid with antioxidant, anti-inflammatory, and

neuroprotective properties (Liu, Meng, Wu, Qiu, & Luo, 2019), was investigated in this study for its therapeutic potential against TAA-induced HE, with particular focus on the gut-microbiota-liver-brain axis.

Our study could establish a model of HE induced by TAA, as evidenced by biochemical, behavioral, and histopathological findings. TAA administration induced significant hepatic injury, demonstrated by marked elevation of serum ALT and AST levels versus the control G. Elevation of these enzymes indicates loss of hepatocyte membrane integrity and leakage of intracellular enzymes into circulation following cellular injury (Kajal, Ganesh, & Sethi, 2023). The hepatotoxic effect of TAA is attributed to its metabolic activation by cytochrome P450 enzymes into reactive metabolites that induce OS, mitochondrial dysfunction, and hepatocyte necrosis (Ezhilarasan, 2023a). TAA administration also resulted in significant elevation of serum and brain ammonia levels, confirming impaired hepatic detoxification capacity. Excess ammonia crosses the BBB and accumulates in astrocytes, leading toglutamine accumulation, astrocyte swelling, and neuronal dysfunction (Gallego-Durán et al., 2024). The successful induction of HE was further confirmed by the passive avoidance test, which showed significant dysfunction in learning and memory functions in TAA-treated rats. Histopathological examination supported these findings, revealing marked disruption of hepatic architecture and pathological alterations in the cerebral cortex and hippocampus. These data are in line with previous investigations (Abd Elrazik & Abd El Salam, 2024; Hajipour, Farbood, Dianat, Nesari, & Sarkaki, 2023a).

Conversely, treatment with BBR significantly decreased serum ALT and AST levels, reflecting attenuation of hepatocellular injury. BBR also significantly decreased serum and brain ammonia levels, suggesting improvement of hepatic detoxification capacity. Moreover, BBR treatment significantly improved cognitive and memory performance in the passive avoidance test, which was confirmed by histopathological examination showing marked improvement of hepatic architecture and reestablishment of normal histological structure in the cerebral cortex and hippocampus. These data are in line with previous investigations revealing that BBR significantly attenuates TAA-induced hepatic injury and hyperammonemia (Ali & Datusalia, 2025; Hajipour, Farbood, Dianat, Nesari, & Sarkaki, 2023b).

The pathogenesis of HE mainly involves the interaction among the gut, liver, and brain, known as the gut-liver-brain axis. Hepatic dysfunction is related with gut dysbiosis and elevated intestinal permeability, facilitating translocation of LPS into the circulation. These endotoxins activate inflammatory signaling pathways through TLR-4, leading to activation of NF-κB pathway and amplification of systemic and neuroinflammation (F. Yang, Gao, Luo, Liu, & Xiong, 2023a). Persistent activation of inflammatory pathways contributes to excessive ROS generation and OS, leading to cellular injury in both hepatic and neural tissues. Alongside with, OS and inflammation disrupt BBB integrity and impair neuronal signaling, which are key pathological features of HE (Di Ciaula, Stella, Bonfrate, Wang, & Portincasa, 2020).

Our study documented that TAA-induced HE was related with a significant increase in serum LPS levels, reflecting endotoxemia resulting from IB disruption. This was supported by histopathological examination of colonic tissue, which revealed marked inflammatory cell infiltration and disruption of normal mucosal architecture. Biochemical analysis showed a marked reduction in intestinal ZO-1 levels, a key tight junction protein essential for maintaining intestinal epithelial barrier integrity (Izadparast, Riahi-Zajani, Yarmohammadi, Hayes, & Karimi, 2022). The decrease in intestinal ZO-1 may be due to the combined impacts of hyperammonemia, OS, and systemic inflammation, which promote degradation of tight junction proteins (Vairappan, Sundhar, & Srinivas, 2019).

Treatment with BBR lead to significant reduction in serum LPS levels, reestablishment of colonic mucosal architecture, and significant increase in intestinal ZO-1 levels. This protective effect may be attributed to berberine's ability to decrease inflammation, inhibit OS, and stabilize tight junction proteins, thereby preventing endotoxin translocation (Cao, Chen, Xu, & Guo, 2022). These data are in line with previous investigations documenting protective impacts of BBR on IB integrity (Li et al., 2011; Yuan et al., 2025). The beneficial impact of BBR on circulating LPS levels may also be attributed to its capability to modulate gut microbiota composition and inhibit bacterial translocation (Izadparast et al., 2022; F. Yang, Gao, Luo, Liu, & Xiong, 2023b).

Moreover, our study documented that serum SCFAs levels showed a significant decline in the TAA-HE G, reflecting gut microbiota dysbiosis and loss of beneficial SCFA-producing bacteria. SCFAs is critical in preserving IB integrity and regulating immune responses (Luo et al., 2023; Z. Zhu et al., 2020). In line with these data, Juanola et al. (Juanola et al., 2019) reported that circulating SCFAs levels were inversely related with hepatic disease severity in patients with cirrhosis. Similarly, Meng et al. (Y. Zhu et al., 2025) demonstrated decreased SCFAs levels in hepatitis B-related acute-on-chronic liver failure. Treatment with BBR lead to a significant raise in serum SCFAs levels, likely via modulation of gut microbiota composition and reestablishment of beneficial SCFA-producing bacteria (F. Yang et al., 2023b). These data are in line with previous investigations (Z.-W. Zhang et al., 2025; Z. Zhang et al., 2016), suggesting that BBR may exert part of its neuroprotective effect through microbial metabolite modulation within the gut-liver-brain axis.

The impaired IB induces persistent endotoxemia with subsequent activation of inflammatory signaling pathways, especially the TLR-4/NF-κB pathway (Luo et al., 2023). The results demonstrated that TAA-induced HE was related with significant activation of inflammatory pathways, as verified by marked raise in hepatic TLR-4 and NF-κB levels. Activation of TLR-4, primarily triggered by LPS, initiates intracellular signaling cascades leading to NF-κB activation and formation of pro-inflammatory CKs, contributing to hepatocellular damage and development of HE (Engelmann, Clària, Szabo, Bosch, & Bernardi, 2021a; Yan et al., 2023). Treatment with BBR significantly reduced hepatic TLR-4 and NF-κB levels through both direct inhibition of NF-κB signaling and indirect reduction of endotoxin load via modulation of gut microbiota and reestablishment of IB integrity (Izadparast et al., 2022; Wang, Zhang, Gao, Zhang, & Gu, 2022). These data are in line with previous investigations (Z. Zhang et al., 2016).

In addition to hepatic inflammation, TAA-induced HE was related with significant neuroinflammation, as evidenced by increased brain TLR-4 and NF-κB levels. This neuroinflammatory response may result from systemic release of inflammatory mediators and endotoxins that disrupt the BBB and activate microglial cells (Engelmann, Clària, Szabo, Bosch, & Bernardi, 2021b). Activation of TLR4/NF-κB signaling in the brain promotes release of proinflammatory CKs and excessive ROS generation, leading to OS and neuronal dysfunction (Yan et al., 2023). This inflammatory activation was accompanied by impairment of BBB integrity, demonstrated by a marked reduction in brain ZO-1 levels (Izadparast et al., 2022; Vairappan et al., 2019). Treatment with BBR significantly decreased brain TLR-4 and NF-κB levels and increased brain ZO-1 levels, reflecting attenuation of neuroinflammation and preservation of BBB integrity. The neuroprotective impact of BBR may be due to both inhibition of inflammatory signaling and stabilization of tight junction proteins, thereby restricting entry of neurotoxic substances into brain tissue (Abd Elrazik & Abd El Salam, 2024; Ali & Datusalia, 2025).

Based on the established link among inflammatory activation and ROS generation, OS status was further investigated. The results revealed that TAA-induced HE was related with significant OS in both hepatic and brain tissues, as evidenced by increased MDA levels and decreased GSH levels. MDA is a determinant of lipid peroxidation, while GSH represents a major intracellular antioxidant. The observed imbalance among pro-oxidant and antioxidant systems contributes to hepatocellular injury and neuronal dysfunction (Gallego-Durán et al., 2024). The induction of hepatic OS by TAA can be explained by its metabolic activation into reactive intermediates that generate excessive ROS (Ezhilarasan, 2023a). At the brain level, hyperammonemia, systemic inflammation, and endotoxin translocation collectively contribute to oxidative imbalance affecting astrocytes and neurons (Gallego-Durán et al., 2024). Treatment with BBR significantly decreased MDA levels and elevated GSH levels in both hepatic and brain tissues, reflecting attenuation of lipid peroxidation and reestablishment of antioxidant capacity. The hepatoprotective effect of BBR may be due to its antioxidant properties, including free radical scavenging and enhancement of endogenous antioxidant systems (Yi et al., 2021). Similarly, the neuroprotective effect may be attributed to enhancement of endogenous antioxidant defenses and stabilization of mitochondrial function (Tian, Sharma, & Dai, 2023). These data are in line with previous investigations (Hajipour et al., 2023b; Kaboutari, Asle-Rousta, & Mahmazi, 2024; Saleh et al., 2021).

Barrier dysfunction and persistent neuroinflammation may subsequently impair neuronal metabolism and neurotransmitter homeostasis, contributing to alterations in glutamatergic signaling (Claeys, Geerts, Van Hoecke, Van Steenkiste, & Vandenbroucke, 2024).Our study documented that TAA-induced HE was related with a significant reduction in brain glutamic acid levels, reflecting disturbance in the glutamate-glutamine cycle. In hyperammonemia, excess ammonia is taken up by astrocytes and converted to glutamine via glutamine synthetase, consuming glutamate and depleting the neuronal glutamate pool (Gallego-Durán et al., 2024; Lu, 2023). Treatment with BBR significantly increased brain glutamic acid levels, likely through reduction of systemic and cerebral ammonia levels, reestablishment of the glutamate-

glutamine cycle, and preservation of neuronal integrity through antioxidant and anti-inflammatory effects (Gallego-Durán et al., 2024). These data are in line with previous investigation (Lemberg & Fernández, 2009; Shurubor, Rogozhin, Isakova, Deryabina, & Krasnikov, 2023).

To our knowledge, simultaneous evaluation of intestinal and brain ZO-1 in berberine-treated TAA-induced HE, together with serum SCFAs, LPS, TLR-4/NF-κB signaling, and behavioral outcomes, has not been comprehensively determined in previous investigations. The novelty of the current work is based on the comprehensive integration of microbial metabolites, endotoxemia, barrier integrity, inflammatory signaling, OS, neurotransmitter disturbance, and cognitive impairment to explain the multi-target protective effect of BBR in TAA-induced HE.

5. Conclusion

TAA successfully induces a comprehensive HE model featuring hepatic injury, hyperammonaemia, neuroinflammation, OS, barrier disruption, and cognitive impairment. BBR demonstrated multi-targeted therapeutic effects, including hepatoprotection, ammonia reduction, gut microbiota modulation, suppression of inflammation and OS, reestablishment of intestinal and BBB integrity, and cognitive improvement. These data emphasize the pivotal role of gut-liver-brain axis in HE pathogenesis and support targeting this axis as a promising therapeutic strategy. However, incomplete normalization of some parameters suggests additional mechanisms may be involved, warranting further research to fully elucidate berberine's therapeutic potential.

Acknowledgment: Not applicable

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Table 1. Comparison of the studied groups as regards behavioral test (passive avoidance test)

	Control group (n= 10)	BBR- treated control group (n= 10)	TAA- HE group (n= 10)	BBR- treated HE group (n= 10)
Time in the dark				
Mean ± SD (seconds)	20.30 ± 4.30	18.70 ± 4.00	96.42 ± 15.50 a, b	30.30 ± 4.51 a, b, c
Latency period				
Mean ± SD (seconds)	96.81 ± 4.22	97.30 ± 4.32	38.71 ± 8.32 a, b	71.60 ± 4.92 a, b, c

One way analysis of variance (ANOVA) test followed by Tukey's post- hoc analysis: a denotes significant differences against control group ($p < 0.05$). b denotes significant differences against BBR- control group ($p < 0.05$). c denotes significant difference against TAA- HE group ($p < 0.05$). BBR- treated control group: Berberine treated control group; TAA-HE group: Thioacetamide induced hepatic encephalopathy group; BBR- treated HE group: Berberine treated hepatic encephalopathy group.

Table 2. Comparison of the studied groups as regards serum and brain biochemicals

	Control group (n= 10) Mean ± SD	BBR- treated control group (n= 10) Mean ± SD	TAA- HE group (n= 10) Mean ± SD	BBR- treated HE group (n= 10) Mean ± SD
ALT (IU/L)	35.70 ± 1.95	33.98 ± 1.68	97.38 ± 1.11 a, b	50.81 ± 2.46 a, b, c
AST (IU/L)	44.97 ± 3.07	45.83 ± 2.83	171.06 ± 6.25 a, b	97.29 ± 5.11 a, b, c
Serum ammonia (mg/dL)	0.84 ± 0.15	0.85 ± 0.22	10.96 ± 1.95 a, b	5.21 ± 1.30 a, b, c
Serum LPS (pg/mL)	28.05 ± 4.22	27.91 ± 4.32	204.76 ± 3.01 a, b	80.31 ± 2.42 a, b, c
Serum SCFAs (mg/dL)	0.72 ± 0.06	0.74 ± 0.06	0.32 ± 0.06 a, b	0.62 ± 0.08 a. b, c
Brain ammonia (µmol/mg tissue)	0.36 ± 0.08	0.34 ± 0.09	2.54 ± 0.39 a, b	1.00 ± 0.12 a, b, c
Brain glutamic acid (µmol/mg tissue)	6.19 ± 1.20	6.28 ± 1.34	1.12 ± 0.19 a, b	3.73 ± 0.53 a, b, c

One way analysis of variance (ANOVA) test followed by Tukey's post- hoc analysis: a denotes significant differences against control group ($p < 0.05$). b denotes significant differences against BBR- control group ($p < 0.05$). c denotes significant difference against TAA- HE group ($p < 0.05$). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LPS: Lipopolysaccharides; SCFAs: Short chain fatty acids.

Table 3: Comparison of the studied groups as regards hepatic, brain inflammatory and oxidative stress markers and barrier integrity marker

	Control group (n= 10) Mean ± SD	BBR- treated control group (n= 10) Mean ± SD	TAA- HE group (n= 10) Mean ± SD	BBR- treated HE group (n= 10) Mean ± SD
Hepatic markers				
TLR-4 (ng/mg protein)	0.35 ± 0.08	0.32 ± 0.09	7.16 ± 0.41 a, b	3.68 ± 0.33 a, b, c
NFκB (ng/mg protein)	0.09 ± 0.03	0.10 ± 0.03	3.09 ± 0.04 a, b	1.29 ± 0.13 a, b, c
MDA (nmol/mg protein)	1.03 ± 0.24	1.06 ± 0.26	5.42 ± 0.64 a, b	2.94 ± 0.42 a, b, c
GSH (nmol/mg protein)	58.54 ± 6.54	57.76 ± 6.96	16.77 ± 3.07 a, b	40.50 ± 3.54 a, b, c
Brain markers				
TLR-4 (ng/mg protein)	0.21 ± 0.06	0.19 ± 0.06	5.02 ± 1.25 a, b	1.49 ± 0.29 a, b, c
NFκB (ng/mg protein)	0.07 ± 0.02	0.07 ± 0.03	2.50 ± 0.31 a, b	0.91 ± 0.29 a, b, c
MDA (nmol/mg protein)	1.04 ± 0.29	1.03 ± 0.26	4.70 ± 0.24 a, b	2.14 ± 0.25 a, b, c
GSH (nmol/mg protein)	40.07 ± 5.38	44.71 ± 7.02	11.65 ± 2.06 a, b	31.51 ± 3.82 a, b, c
Brain markers				
Brain ZO-1 (units)	0.84 ± 0.09	0.86 ± 0.08	0.09 ± 0.01 a, b	0.30 ± 0.07 a, b, c
Intestinal ZO- 1 (units)	1.24 ± 0.16	1.25 ± 0.15	0.39 ± 0.06 a, b	0.84 ± 0.09 a, b, c

One way analysis of variance (ANOVA) test followed by Tukey's post- hoc analysis: a denotes significant differences against control group ($p < 0.05$). b denotes significant differences against BBR- control group ($p < 0.05$). c denotes significant difference against TAA- HE group ($p < 0.05$). TLR- 4: Toll- like receptor- 4; NFκB: Nuclear factor kappa binding protein; MDA: Malondialdehyde; GSH: glutathione; ZO-1: Zona occludens- 1