

Original paper

Taurine supplementation abates cirrhosis-associated locomotor dysfunction

Reza Heidari¹, Akram Jamshidzadeh¹, Vahid Ghanbarinejad¹, Mohammad Mehdi Ommati², Hossein Niknahad¹

¹Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

²Department of Animal Sciences, School of Agriculture, Shiraz University, Shiraz, Iran

Abstract

Aim of the study: Hepatic encephalopathy and hyperammonemia is a clinical complication associated with liver cirrhosis. The brain is the target organ for ammonia toxicity. Ammonia-induced brain injury is related to oxidative stress, locomotor activity dysfunction, and cognitive deficit, which could lead to permanent brain injury, coma and death if not appropriately managed. There is no promising pharmacological intervention against cirrhosis-associated brain injury. Taurine (TAU) is one of the most abundant amino acids in the human body. Several physiological and pharmacological roles have been attributed to TAU. TAU may act as an antioxidant and is an excellent neuroprotective agent. This study aimed to evaluate the effect of TAU supplementation on cirrhosis-associated locomotor activity disturbances and oxidative stress in the brain.

Material and methods: Rats underwent bile duct ligation (BDL) surgery, and plasma and brain ammonia level, plasma biochemical parameters, and rats' locomotor function were monitored. Furthermore, brain tissue markers of oxidative stress were assessed.

Results: It was found that plasma and brain ammonia was increased, and markers of liver injury were significantly elevated in the cirrhotic group. Impaired locomotor activity was also evident in BDL rats. Moreover, an increase in brain tissue markers of oxidative stress was detected in the brain of cirrhotic animals. It was found that TAU supplementation (50, 100, and 200 mg/kg, gavage) alleviated brain tissue markers of oxidative stress and improved animals' locomotor activity.

Conclusions: These data suggest that TAU is a potential protective agent against cirrhosis-associated brain injury.

Key words: amino acid, hepatic encephalopathy, hyperammonemia, neurotoxicity, oxidative stress.

Address for correspondence

Reza Heidari, Shiraz University of Medical Sciences, P. O. Box 1583; 71345. Roknabad, Karafarin St., P. O. Box 1583; 71345 Shiraz, Fars, Iran, phone: +98 9171237882, e-mail: rezaheidari@hotmail.com

Introduction

Hepatic encephalopathy (HE) and hyperammonemia is a clinical feature of chronic liver injury and cirrhosis [1]. Although the exact mechanism(s) of HE-associated complications is not known, there is agreement on the predominant role of ammonia [2]. Typically, ammonia is metabolized to urea by the liver. When the liver is damaged (e.g., by diseases or xenobiotics), this organ is not able to detoxify ammonia. The brain is a crucial target organ for ammonia toxicity. Several mechanisms have been proposed for ammonia-induced

neurotoxicity [3, 4]. It has been found that ammonia has direct toxic effects on neurons and astrocytes [4]. Ammonia causes brain edema, neuroinflammation, and oxidative stress when its level rises during HE [5]. Consequently, suppression of the brain function, coma, permanent brain injury, or death might occur in patients with HE [5]. Impaired locomotor activity and cognitive dysfunction are well-established symptoms of ammonia neurotoxicity [6, 7]. Suppression of brain function during HE and hyperammonemia could lead to coma and death if not appropriately managed [6, 7]. Altered motor function in patients with chronic HE

and hyperammonemia could reduce the quality of life of cirrhotic patients.

Oxidative stress and its associated complications are known to be implicated in ammonia-induced brain injury [3, 8, 9]. It has been reported that ammonia caused severe oxidative/nitrosative stress, biomembrane disruption, lipid peroxidation, and defects in cellular antioxidant systems in the brain tissue [10-15]. Hence, antioxidants and protective agents might have therapeutic value in the management of hyperammonemia-associated brain injury and its associated complications.

Taurine (2-aminoethane sulfonic acid; TAU) is one of the most abundant amino acids in the human body [16]. Although TAU is not incorporated in protein structures, several physiological and pharmacological properties are attributed to this amino acid [17-21]. The cytoprotective properties of this chemical have been widely investigated [22-27]. The therapeutic effect of TAU against several diseases has also been mentioned [28-30]. It has been found that TAU provides protection against several neurological disorders as well as xenobiotic-induced neurotoxicity [31-36].

Although the protective properties of taurine have been widely investigated, the effect of this chemical against hyperammonemia-associated complications such as impairment of locomotor activity and brain injury has not been entirely revealed. In the current study, bile duct ligation (BDL) was used as a reliable animal model of cirrhosis [37, 38]. Then, plasma biomarkers of liver injury were assessed. Moreover, brain tissue oxidative stress markers were measured, and animals' locomotor activity was monitored to investigate the effect of TAU supplementation on cirrhosis-associated brain injury and impairment of locomotor activity.

Material and methods

Chemicals

Fatty acid-free bovine serum albumin (BSA) fraction V, dithiobis-2-nitrobenzoic acid (DTNB), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), 4,2-hydroxyethyl,1-piperazine ethane sulfonic acid (HEPES), thiobarbituric acid (TBA), 2',7'-dichlorofluorescein diacetate (DCFH-DA), taurine (TAU), malondialdehyde (MDA), glutathione (GSH), sodium phosphate dibasic (Na_2HPO_4), sucrose, potassium chloride (KCl), Coomassie brilliant blue, dithiothreitol, ethylene glycol-bis (2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydroxymethyl aminomethane hydrochloride (Tris-HCl), and trichloroacetic acid

(TCA) were obtained from Merck (Darmstadt, Germany). All salts (analytical grade) for preparing buffer solutions were obtained from Merck (Darmstadt, Germany).

Animals

Male Sprague Dawley rats ($n = 48$; 200-250 g weight) were obtained from the Animal Breeding Center, Shiraz University of Medical Sciences, Shiraz, Iran. Rats were housed in plastic cages over wood-chip bedding (ambient temperature of $23 \pm 1^\circ\text{C}$, 12L: 12D photo schedule, $\approx 40\%$ of relative humidity). Animals were allowed free access to a standard chow diet (Behparvar, Tehran, Iran) and tap water. All the experiments were conducted in conformity with the guidelines for care and use of experimental animals approved by the local ethics committee of Shiraz University of Medical Sciences, Shiraz, Iran (#14822).

Animal model of cirrhosis

Bile duct ligation (BDL) in rats is an animal model of cirrhosis with all complications of chronic HE including a rise in blood ammonia and its associated neurobiological complications [14, 39]. For BDL surgery, animals were anesthetized (10 mg/kg of xylazine and 70 mg/kg of ketamine, i.p.), a midline incision was made, and the common bile duct was identified, doubly ligated, and cut between these two ligatures [40]. The sham operation comprised laparotomy and bile duct identification and manipulation without ligation.

Animal treatments

TAU (dissolved in tap water) was administered orally (Gavage) for 28 consecutive days. The treatments were as follows: 1) Control (vehicle-treated; tap water 2 ml/kg); 2) BDL; 3) BDL + TAU 50 mg/kg/day, oral; 4) BDL + TAU 100 mg/kg/day, oral; 5) BDL + TAU 200 mg/kg/day, oral; 6) TAU 200 mg/kg/day, oral. On day 29 after the BDL operation, animals' locomotor activity was assessed. Then, animals were anesthetized (thiopental, 80 mg/kg, i.p.) and samples were collected. The sole TAU (200 mg/kg, oral) was administered to ensure its safety.

Motor coordination and activity tests

All motor coordination and activity tests were conducted on day 29 after BDL surgery.

Open field test behavior

Open field behavior is applied as an index of animals' locomotor activity in animal models of HE [41, 42]. In the

current investigation, the open field apparatus was made of a white Plexiglas box (100 cm L × 100 cm W × 30 cm H, and the box floor was divided into squares of 10 × 10 cm) [43]. The open field arena was equipped with a webcam (2.0 Megapixel, Gigaware, UK) and animals activity was monitored and recorded from a separate room. Rats' behavior was recorded for fifteen minutes, and the total number of crossed squares was counted (total locomotion) [44].

Rotarod test

Based on a previously reported procedure, each rat underwent five sessions of rotarod performance [45]. The speed of the rotarod was 5 and 15 rpm with a cut-off point 300 s. The time up to which the rat stayed on the rotating rod was automatically recorded [45, 46].

Gait test

Animals' hind paws were wetted with ink. Afterward, using a runway procedure, rats were allowed to walk down on a paper strip (60 cm long, 10 cm wide) from the brightly lit corridor toward a dark side. The distance between the points of the left and right hind paws was measured and recorded [45].

Beam walk

Animals had to cross a beam (15 mm diameter; 80 cm long; elevated 50 cm over the ground). The beam communicated with a box at one end. Animals were first trained with a series of three approximate trials. Then, the time of beam cross and the number of foot slips were recorded [47].

Adhesive-removal test

The adhesive removal test was performed to evaluate animal' sensorimotor impairment, [48, 49]. A small adhesive-back paper dot (8-mm diameter) was placed on the rat forepaw to cover the hairless part of the paw. The animal was placed in a box (40 cm L × 40 cm W × 15 cm H) and the time to remove the strip (with a cut-off point of 180 s) was recorded [48, 49].

Negative geotaxis test

Based on a previously reported procedure, rats were placed on an inclined surface (30°) with their heads facing downward [50]. The time for each animal to turn 180° was measured with a cut-off point of 90 s [50].

Blood biochemistry

A Mindray BS-200 autoanalyzer (Mindray chemistry analyzers for low-volume laboratories, Guangzhou, China) and standard commercial kits (Pars Azmun, Tehran, Iran) were used to measure serum albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) [51]. Plasma ammonia level was measured with standard kits based on the absorbance photometry method of phenate-hypochlorate reaction [52]. Brain ammonia level of cirrhotic animals was determined according to a previously reported method [44] (Table 1). Briefly, forebrain (cerebral cortex) samples (100 mg) were dissected, homogenized, and deproteinized in 3 ml of an ice-cooled (4°C) lysis solution (trichloroacetic acid, 6% w/v in double distilled water). After centrifugation (12,000 g, 10 minutes, 4°C), the supernatant was collected and neutralized (KHCO₃; 2 mol/l, pH = 7). Afterward, the ammonia content of the supernatant was measured using standard kits [52].

Statistical analysis

Data are shown as mean ± SD. The comparison of data sets was performed by the one-way analysis of variance (ANOVA) and Tukey's post hoc test. Differences between groups were considered statistically significant when $p < 0.05$.

Results

Liver cirrhosis in BDL rats was accompanied with severe changes in blood biochemistry as compared with the sham-operated group. On the other hand, it was found that TAU treatment (50, 100, and 200 mg/kg/day, oral) decreased serum biomarkers of liver injury in cirrhotic animals. A higher level of ammonia was detected in the plasma of BDL rats. Brain tissue ammonia level was also significantly higher in cirrhotic animals in comparison with the sham-operated group. It was found that both plasma and brain ammonia level was lower in TAU-supplemented animals (200 mg/kg/day, oral).

Evaluation of animals' motor coordination revealed a significant decrease of locomotor activity in BDL rats. Lower open field activity, and impaired rotarod, beam walk, and gait test were evident in cirrhotic animals. The adhesive removal test as an index of sensorimotor activity was also impaired in the BDL group. It was found that TAU supplementation (50, 100, and 200 mg/kg/day, oral) significantly decreased

Table 1. Serum biochemical measurements

Biomarkers assessed	Treatments				
	Control (vehicle-treated)	BDL	BDL + TAU 50 mg/kg	BDL + TAU 100 mg/kg	BDL + TAU 200 mg/kg
ALT (U/l)	51 ± 4	430 ± 25*	259 ± 60 ^a	183 ± 61 ^a	164 ± 65 ^a
AST (U/l)	83 ± 15	302 ± 21*	250 ± 27 ^a	116 ± 47 ^a	102 ± 35 ^a
LDH (U/l)	402 ± 79	917 ± 96*	688 ± 43	512 ± 30 ^a	593 ± 24 ^a
Total bilirubin (mg/dl)	0.054 ± 0.005	11 ± 2*	7 ± 3	6.5 ± 1.8	6.4 ± 3.47
Albumin (mg/dl)	3.66 ± 0.22	2.95 ± 0.41*	3.00 ± 0.22	3.19 ± 0.12 ^a	3.30 ± 0.11 ^a
Plasma ammonia (mg/dl)	237 ± 66	1262 ± 214*	969 ± 160	898 ± 239	827 ± 217 ^a
Brain ammonia (mg/g tissue)	11 ± 2	41 ± 9*	32 ± 7	27 ± 10	23 ± 7 ^a

Data are shown as mean ± SD (n = 8).

*Indicates significantly different as compared with control group (p < 0.001).

^aIndicates significantly different as compared with BDL group (p < 0.05).

BDL – bile duct ligated, TAU – taurine

the impairment of animals' locomotor activity in BDL rats (Fig. 1).

It was found that markers of oxidative stress were significantly higher in the brain tissue of cirrhotic rats. A high level of reactive oxygen species (ROS), along with tissue glutathione depletion, and severe lipid peroxidation, were detected in the brain tissue of the BDL group in comparison with sham-operated animals. Moreover, the antioxidant capacity of the brain tissue was significantly decreased in cirrhotic rats. It was found that TAU treatment (50, 100, and 200 mg/kg, i.p.) significantly mitigated brain tissue biomarkers of oxidative stress in cirrhotic animals. Lower levels of ROS and lipid peroxidation were detected in TAU-supplemented groups. TAU (50, 100, and 200 mg/kg, i.p.) also preserved tissue antioxidant capacity and prevented brain glutathione depletion (Fig. 2).

It is noteworthy that the sole TAU administration caused no significant changes in animals' locomotor activity in comparison with the control (vehicle-treated) group (data not shown). On the other hand, markers of oxidative stress remained unchanged (except for glutathione content, which was higher; p < 0.05) in the brain tissue of TAU-treated animals in comparison with the control group (data not shown). On the other hand, the effect of different doses of TAU (50, 100, and 200 mg/kg/day, oral) on animals' locomotor activity and brain markers of oxidative stress was not dose-dependent.

Discussion

Chronic hepatic encephalopathy (HE) and hyperammonemia is a common event associated with cirrhosis [53, 54]. HE is a neuropsychiatric syndrome

which can lead to permanent brain injury, coma, and death if not appropriately managed [1, 5, 6]. On the other hand, chronic and frequently subclinical hyperammonemia is associated with different degrees of cirrhosis and could affect patients' CNS function and quality of life [55, 56]. A wide range of impaired psychomotor performance including tremor, rigidity, akinesia, athetosis, as well as cognitive dysfunction, is associated with cirrhosis and chronic HE [1, 57-59].

Ammonia is the most suspected molecule involved in the pathogenesis of HE-induced brain injury [2]. Oxidative stress and its consequences are established to play a significant role in the pathogenesis of hyperammonemia-induced brain injury [9-12, 60-62]. In the current study, it was revealed that TAU supplementation (50, 100, and 200 mg/kg, i.p.) to cirrhotic rats recovered animals' regular locomotor activity and alleviated brain tissue markers of oxidative stress.

The neuroprotective properties of TAU have been widely investigated [63]. It is well established that taurine treatment efficiently encounters oxidative stress and its consequences in brain tissue [64-66]. Several neurological disorders have also been shown to benefit from TAU supplementation [31-36, 67]. The effects of this amino acid against xenobiotic-induced CNS injury have also been widely investigated [68, 69].

Oxidative stress and its associated events play a central role in ammonia-induced neurotoxicity [12, 70]. It has been found that markers of oxidative stress were increased in the brain tissue of cirrhotic animals [71-73]. Increased ROS level, severe lipid peroxidation, and decreased brain tissue glutathione stores were detected in the brain of cirrhotic animals (Fig. 2) [74]. Brain tissue antioxidant capacity was also impaired in BDL rats (Fig. 2). In the current study, TAU (50, 100, and 200 mg/kg, i.p.)

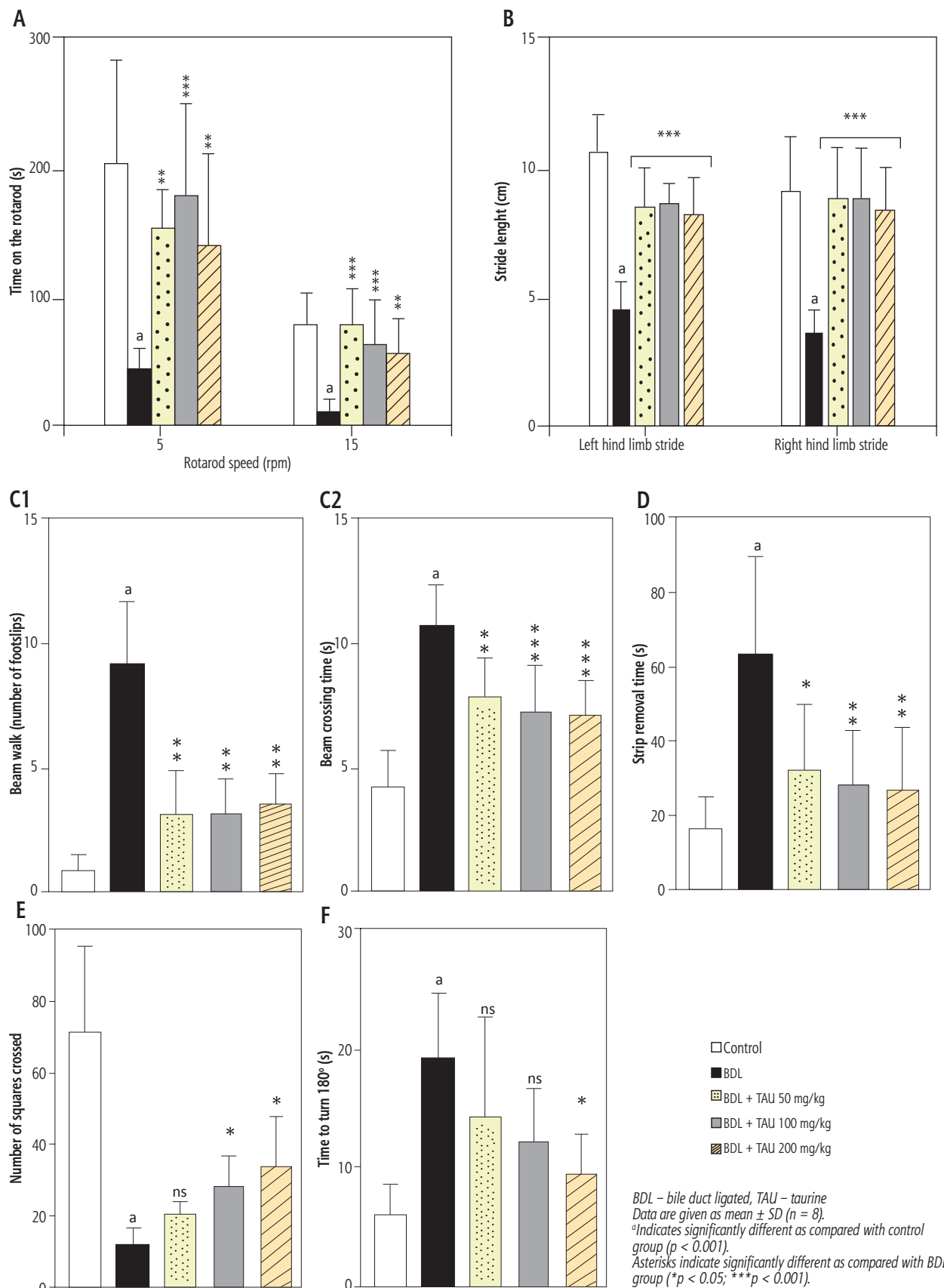
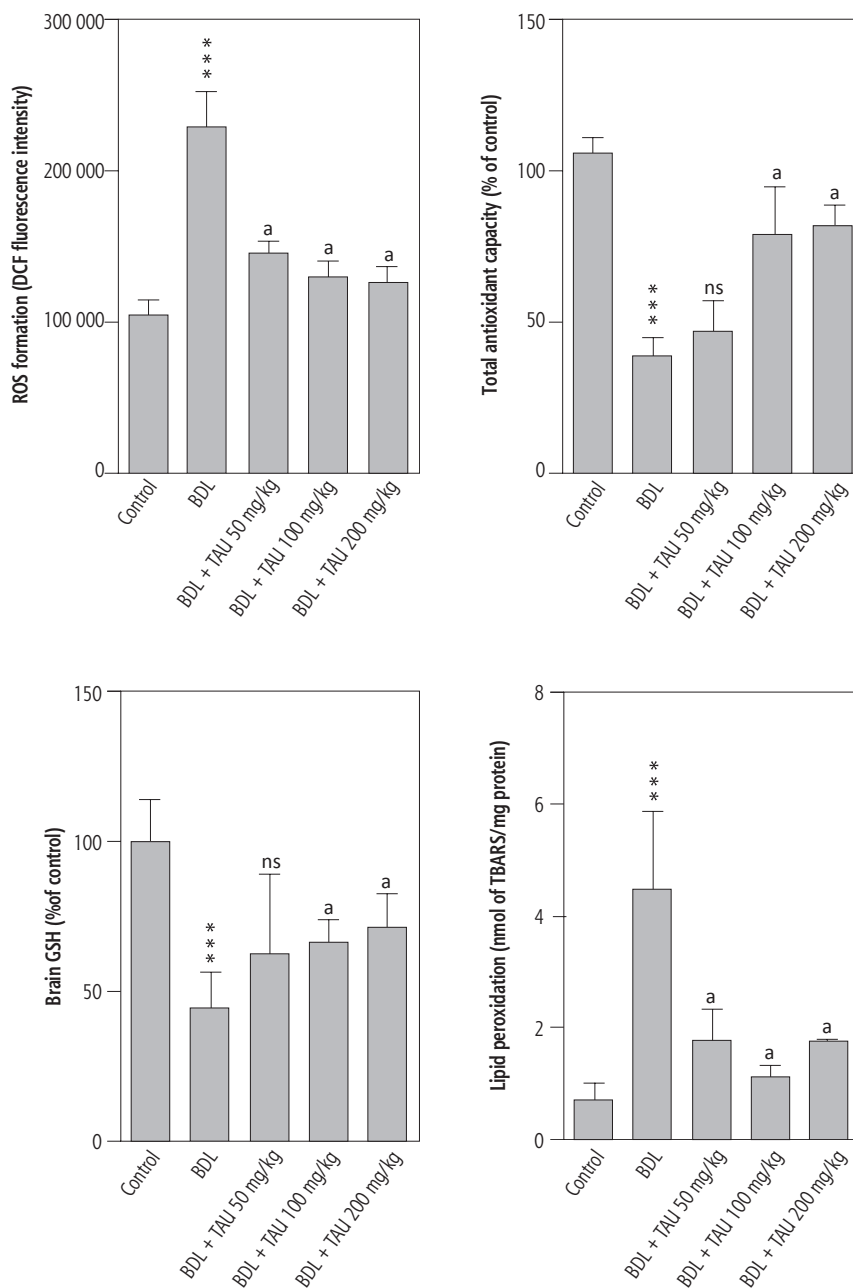


Fig. 1. Effect of taurine (TAU) administration on the animals' locomotor activity in bile duct ligated (BDL) rat model of cirrhosis. **A)** rotarod test, **B)** gait test, **C1** and **C2)** beam walk activity, **D)** adhesive-removal test, **E)** open field behavior; and **F)** negative geotaxis test



***Indicates significantly different as compared with control group ($p < 0.001$).

^aIndicates significantly different as compared with BDL group ($p < 0.001$).

ns – not significant as compared with BDL group ($p > 0.05$).

BDL – bile duct ligated, TAU – taurine

Fig. 2. Effect of taurine supplementation on brain tissue markers of oxidative stress in cirrhotic animals

effectively alleviated oxidative stress and its consequences in the brain of cirrhotic animals. Previously we also found that TAU treatment alleviated brain tissue markers of oxidative stress in an acute liver failure animal model of hyperammonemia [75]. It has been reported that TAU could significantly mitigate oxidative stress and its related events in different experimental models including several neurological disorders [24, 76]. Hence, the antioxidant

capacity of TAU might play a significant role in its neuroprotective properties during liver failure.

Brain mitochondria are among major targets of ammonia toxicity [77, 78]. It has been established that hyperammonemia leads to a brain energy crisis [77, 78]. Previously we found that TAU could preserve brain mitochondrial function in hyperammonemic conditions [79, 80]. Hence, another dominant mechanism for the

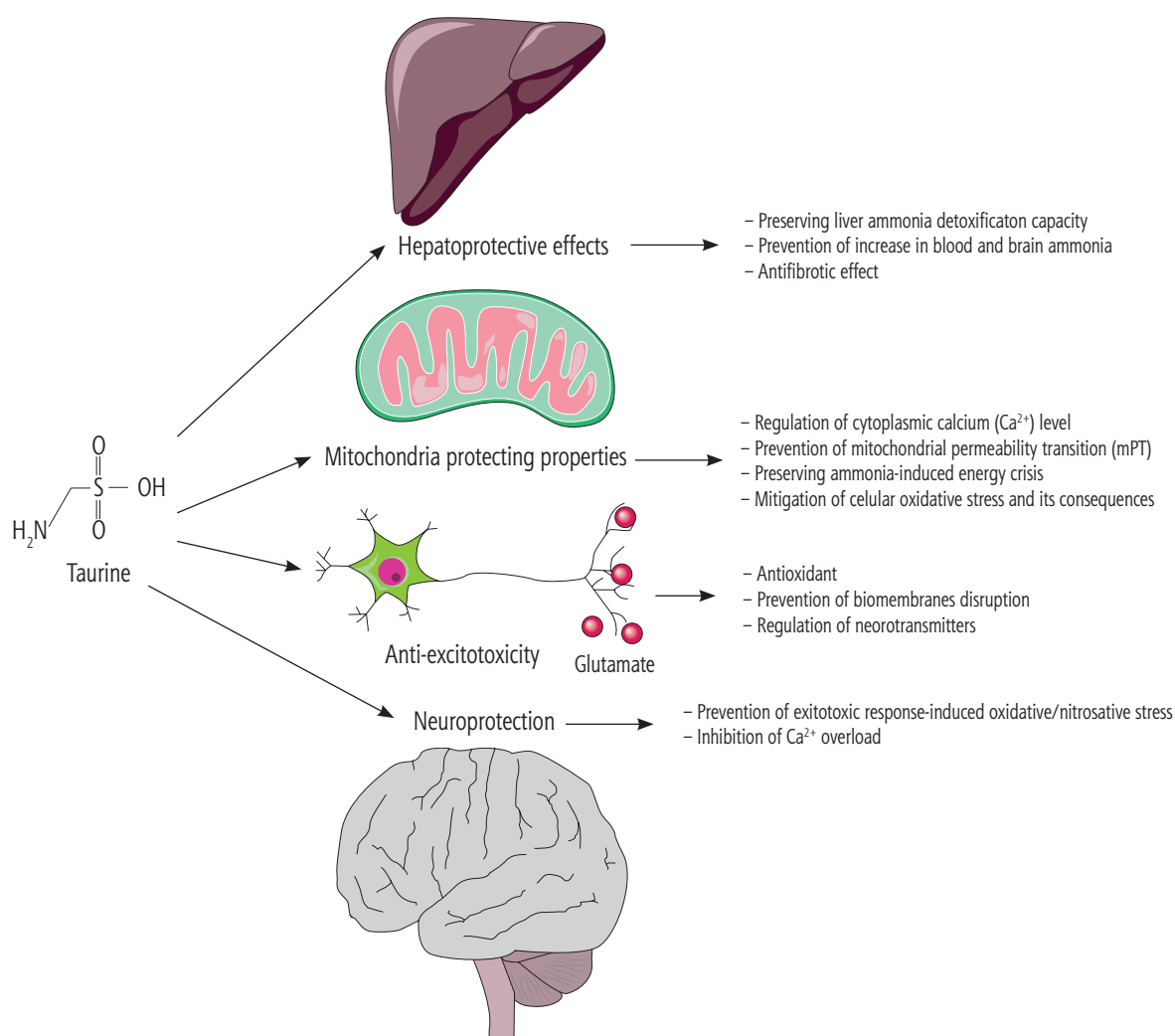


Fig. 3. Schematic representation of the potential mechanisms of neuroprotection provided by taurine in cirrhotic rats. Taurine might protect against ammonia neurotoxicity through a series of interconnected mechanisms

neuroprotective effects of TAU in cirrhosis could be mediated through its impact on cellular mitochondria. Interestingly, some investigations have also mentioned that the anti-oxidative stress effects of TAU might be mediated through its effects on cellular mitochondria [81-84]. Hence, another important mechanism for the neuroprotective properties of TAU in cirrhotic animals could be mediated through its effects on brain mitochondria. The impact of TAU supplementation on brain mitochondrial function and energy metabolism in cirrhosis deserves further investigations.

Impaired cycling of Gln-Glu between neurons and astrocytes is documented in the brain of hyperammonemic models [85]. Consequently, the extracellular concentration of glutamate is increased. Glutamate is the primary excitatory neurotransmitter in the CNS which activates the N-methyl aspartate (NMDA) type of glutamate receptors. Hence, brain glutamatergic

neurotransmission is severely affected during hyperammonemia and HE [86, 87]. It is well established that one of the leading contributors to the toxic effects of ammonia in the brain tissue is the over-activation of NMDA receptors [87, 88]. This over-activation is known as the ammonia-induced “excitotoxic” response [87, 88]. It has been found that the “excitotoxic response” plays a significant role in the pathogenesis of ammonia-induced brain injury [87, 88]. Deleterious events such as dysregulation of cytoplasmic calcium level and excessive formation of reactive oxygen/nitrogen species might lead to NMDA receptor over-activation [5, 70]. Hence, the excitotoxic response is tightly linked to ammonia-induced oxidative/nitrosative stress in the CNS. The antiexcitotoxic effect of TAU is an essential feature of this amino acid [89, 90]. It has been shown that TAU mitigated the excitotoxic response in cultured neurons [91, 92]. Hence, the anti-

excitotoxic effects of this amino acid could also play a role in its neuroprotective effects during hyperammonemic episodes.

Neuroinflammation is another major complication during hyperammonemia and HE [93-95]. It has been found that neuroinflammation during hyperammonemia significantly deteriorates locomotor activity [96]. On the other hand, the anti-inflammatory effect of TAU has been mentioned in several investigations [97-100]. Hence, this amino acid might also alleviate brain tissue inflammation in hyperammonemic animals. The effect of TAU on brain inflammation in different models of hyperammonemia could be the subject of future studies.

We previously found that TAU administration to chronic and acute liver failure animal models could prevent a rise in blood and brain ammonia level [79, 101]. The effect of TAU on ammonia level could be due to the direct effects of TAU on the liver and preserved ammonia detoxification capability of this organ. Hence, the hepatoprotective effects of TAU might also play a significant role in the neuroprotection provided by this amino acid (Fig. 3). In the current study, we found that TAU supplementation efficiently mitigated blood and brain ammonia level as well as impairment in animals' locomotor activity during cirrhosis. Furthermore, TAU treatment prevented ammonia-induced oxidative stress and its consequences in rat brain. All these data indicate TAU as a potentially safe and clinically applicable agent against HE and its associated complications in humans.

Interestingly, it has been found that brain TAU level is changed during acute or chronic HE [102]. Hence, some investigations have mentioned a potential role of TAU in the pathogenesis of HE. It has also been found that TAU prevented bilirubin-induced neurotoxicity [68, 103]. As chronic liver failure and cirrhosis are associated with high bilirubin levels, part of the neuroprotection provided by taurine in BDL animals might be mediated through its effect on bilirubin-induced CNS injury. The precise effects of TAU supplementation on bilirubin-induced neurotoxicity during cirrhosis need further research.

Collectively, the data presented in the current study suggest that TAU exhibits neuroprotective effects against impairment of locomotor activity and oxidative stress associated with cirrhosis. Hence, TAU supplementation could be not only an excellent hepatoprotective strategy but also a potential therapeutic option against hyperammonemia-associated CNS complications. Indeed, further investigations are needed for understanding the effect of TAU supplementation on other critical aspects of HE such as brain edema.

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Disclosure

Authors report no conflict of interest.

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