WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 9, 113-131 Research Article ISSN 2278 – 4357

BIOCHEMICAL AND PATHOPHYSIOLOGICAL ASPECTS OF THE TREATMENT OF HEPATIC ENCEPHALOPATHY IN RATS

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Article Received on
05 July 2017,
Revised on 25 July 2017,
Accepted on 15 August 2017,
DOI: 10.20959/wjpps20179-10047

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ABSTRACT

Hepatic encephalopathy (HE) represents a diverse spectrum of complex neuropsychiatric disturbance resulting from liver disease and its concomitant metabolic and immunologic derangements. Hyperammonemia and inflammation cooperate to induce neurological alteration in hepatic encephalopathy. This study was conducted to elucidate the changes of serum, liver and brain proinflammatory cytokines in encephalopathy rats and to evaluate the hepato-protective activity of lactulose and/or taurine against thioacetamide (TAA) induced hepatic encephalopathy in rats. Acute hepatic encephalopathy (HE) in rats was induced by intraperitoneal injection of thioacetamide in 24 hours intervals for two consecutive days. The obtained results revealed a significant ($p<0.05$) increase in the levels of serum liver function tests (ALT, AST, ALP & bilirubin) in TAA rats group than those in control ones. In addition, the levels of ammonia and pro-inflammatory cytokines (IL-6, IL-1β & TNF-α) in serum, liver and brain were significantly ($p<0.05$) increased associated with a remarkable elevation in the level of serum S100β in TAA rats group. On the other hand, induction of hepatic encephalopathy caused a significant ($p<0.05$) decrease in albumin and total protein levels than those in control rats. When HE rats group was treated with lactulose or taurine and their mixture a considerable amelioration effects in all previous studied parameters were pronounced dependent on certain mechanisms.

KEYWORDS: Hepatic encephalopathy - Cytokines - S100β - Lactulose - Taurine - Rats.

1. INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome which associated with liver dysfunction and has quantitatively and qualitatively distinct features relating to its severity.[1] The pathogenesis of HE is not clearly known, while, it is generally held that hyperammonemia plays the chief causative factor for both direct and indirect alteration in cerebral mechanism.[2] Under normal physiological conditions, endogenous or exogenous ammonia are detoxified in the liver. When the liver fails, ammonia rapidly accumulates in the circulation, freely crosses the blood-brain barrier and accumulates in the brain; result in astrocyte swelling, even death.[3]

Other mechanisms have been proposed to explain the pathogenesis of HE, including dysfunction of the immune and neurotransmitter systems. Also, inflammation plays a significant role in the molecular pathogenesis of HE and brain edema.[4] **Chu et al., (2001)**[5] demonstrated a significant correlation between plasma levels of tumor necrosis factor-α and the severity of HE in rats with thioacetamide-induced hepatic failure. Circulating proinflammatory cytokines correlate with encephalopathy regarding brain edema in acute liver failure and ammonia. However, no conclusive evidence has been obtained for increasing brain proinflammatory cytokines production in HE.

S100β is a member of the S100 family of proteins that was termed “S100” because it was soluble in 100% saturated ammonium sulfate solution. S100β is produced primarily by astrocytes and exerts autocrine and paracrine effects on glia, neurons and microglia.[6] Also, S100β has a neuro-protective function in enhancing neuronal cell maintenance, preventing motor neuron degeneration and enhancing the survival of neurons.[7] Normally, S100β is low or undetectable in serum; however, elevation of serum S100β levels has been detected in a number of neuropathological conditions. It causes neurotoxicity at relatively high concentrations (micromolar doses).[8]

Lactulose is a synthetic and non-digestible sugar used in the treatment of chronic constipation and hepatic encephalopathy. It is a disaccharide (double sugar) formed from one molecule each of the simple sugars (monosaccharide) fructose and galactose. Lactulose passes unchanged into the colon where it is hydrolysed by bacterial action to organic acids, principally acetate and lactate.[9] Proposed mechanisms of action are lowering colonic pH, thereby decreasing the production of ammonia by bacteria and the absorption of non-ionized ammonia.[10] **Luo et al. (2015)**[11] indicated that non-absorbable disaccharides not only reduce

circulating levels of ammonia but also decrease those of pro-inflammatory cytokines and endotoxin.

Taurine (β-amino sulphonic acid) is one of the most abundant amino acid in the human body.[12] Taurine is a derivative of the sulfur-containing amino acid, cysteine. It has been reported that the hepatic taurine level decreased in some liver disease and/or xenobiotics induced hepatic injury.[13] Concentration of taurine in plasma and urine is also reported as a biomarker of hepatic injury because of its release from pre-centeral hepatocytes. Furthermore, taurine plays a protective role against many xenobiotics.[14] Therefore, taurine serves as a hepato-protective agent to prevent liver injury. Also, it has been shown that taurine acts as an osmoregulator, protect neurons, prevent astrocytes swelling and encounters oxidative stress in CNS.[15]

The current investigation focused on the relation between pathogenesis of hepatic encephalopathy induced with thioacetamide (TAA) associated with a remarkable elevation in both cytokines and S100ß levels in rats. Also, this study aimed to assess the possible ability of lactulose or taurine and their mixture in limiting inflammation and disturbance in an experimental model of hepatic encephalopathy rats.

2. MATERIALS AND METHODS

One hundred and twenty adult male albino rats (*Rattus rattus*) (120±10g) were employed in this study. They were housed under controlled environmental conditions (12/12h light/dark cycle and 24^{o}C) in a well-ventilated Vivarium at Biological Applications Department, Nuclear Research Center, Atomic Energy Authority. The animals fed on a standard diet.[16] Food and tape water were served *ad libitum* with fresh supplies presented daily.

This study was included two experiments: In the first experiment, hepatic encephalopathy was induced in 90 rats after one week on basal diet by intraperitoneal injection of thioacetamide (TAA) (Sigma Chem. Co., St Louis, Mo. USA) at dose of 300mg (dissolved in normal saline)/kg.b.wt./day for two consecutive days.[17] To avoid hypoglycemia and electrolytes imbalance, 0.5ml of 10% glucose water mixed with 0.5ml of lactate Ringer solution (25ml/kg) was injected subcutaneously every 12 hours after the first injection of thioacetamide (TAA) for one day.[18] Control rats (30 rats) were injected with normal saline (0.9%NaCl). After one week, ten rats from each previous group were taken to compare the alteration which occurred due to induction of hepatic encephalopathy in rats.

In the second experiment (100 rats), four comparisons were made between normal control rats (20 rats) and four equal subgroups of rats with experimentally hepatic encephalopathy (80 rats). The first experimentally hepatic encephalopathy subgroup rats was not further treated and served as recovery group. The second hepatic encephalopathy subgroup rats were received orogastric dose of 20ml lactulose (Egyptian International Pharmaceutical Industries Co., EIPICO)/ kg.b.wt twice daily with the aid of gastric tube according to **Lin & Zhang (2005)**[19] and served as lactulose rats subgroup. The third hepatic encephalopathy subgroup rats were injected intraperitoneally with 500mg taurine (Sigma Chem. Co., St Louis, Mo. USA) kg.b.wt./day according to **Chen *et al.* (2006)**[20] with a little modification by **Xiao *et al.* (2008)**[21] and served as taurine animals subgroup. The fourth hepatic encephalopathy subgroup rats were received the same dose of both lactulose and taurine as previous described. Control and all animal subgroups were divided into four intervals (1, 2, 4 & 8 weeks; five rats in each interval). At the end of each experimental period, rats were overnight fasted, anaesthetized by diethyl ether in dry clean test tubes. Sera were separated and kept at -20^{o}C for the determination of biochemical parameters.

After sacrifice, livers were removed aseptically at the end of each experimental period. They washed with saline solution (0.9% NaCl) and kept at -20°C till investigations. Also, cortical brain tissues were collected on cooled plate and wash gently in cool saline (0.9% NaCl) at different time-points (1,2,4&8 weeks). Then, tissues were homogenized in two volumes of 0.001 mol/L phosphate-buffered saline (PBS) containing 0.05% Tween-20. After homogenization and centrifugation at 10,000g at 4^{o}C for 20 minutes, the resultant supernatant was collected and stored at-80^{o}C until use.

2. A. Biochemical Analysis

The activities of serum enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] and the concentration of ammonia, total protein, albumin and bilirubin were determined colourimetrically using commercial kits purchased from DIACHEM Ltd., Budapest, Hungary.

The levels of rat interleukin-1ß (rat IL-1ß), interleukin-6 (rat IL-6) and tumour necrosis factor-alpha (TNF-α)were measured in serum, liver and brain using ELISA kits specific for rats purchased from Immuno-Biological Laboratories (IBL) Co., Ltd., USA. However, The serum level of rat soluble protein-100β (S100β) was assayed by the aid of ELISA (Sandwich immunoassay technique) using commercial kit (CUSABIO, China).

2. B. Statistical Analysis

Statistical differences between the means were assessed by analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS version 15.

3. RESULTS AND DISCUSSION

Hepatic encephalopathy (HE) is a morbid neuropsychiatric complication resulting from decompensated liver disease or portosystemic shunting and is characterized by disturbance of both cognitive and motor functions, ranging from subtle psychometric abnormalities to coma.[22] In the current study, TAA administration caused significant ($p<0.05$) increase in the activities of ALT, AST & ALP associated with a remarkable elevation in the concentration of bilirubin and ammonia in experimental animal group compared with those obtained in their corresponding control animal group (Table 1). These results were in accordance with previous studies.[23&24] The authors reported that elevation of the activity of liver enzymes due to TAA administration indicates the cellular leakage and loss of functional integrity of the cell membrane in liver.[25]

Following TAA administration, it undergoes an extensive metabolism by microsomal CYP2E1 to acetamide and thioacetamide-S-oxide which causes disturbance in cell permeability, elevation in both intracellular Ca^{+2} concentration and nuclear volume associated with enlargement of nucleoli and inhibition of mitochondrial activity.[26] Thioacetamide-S-oxide is further metabolized, at least in part by cytochrome-P-450 monooxygenase to further products, including a polar product sulfene, thioacetamide-S-dioxide, a very reactive compound.[27] The binding of this metabolite to tissue macromolecules may be responsible for the production of hepatic necrosis, hyperammonemia and oxidative stress.[28] In addition, the administration of TAA led to excessive in the level of bilirubin may be due to the pronouncing of destruction in hepatic cells accompanied with inhibiting incorporation of amino acids into liver protein.[29]

Table (1): Changes in biochemical parameters and cytokines in serum, liver and brain after TAA administration (Mean ± SE).

Parameters		Mean ± SE		% of change	P Value
		Control	Hepatic encephalopathy (HE)		
Serum	ALT (U/L)	30.33 ± 1.14	50.26 ± 0.98	65.7	<0.05
	AST (U/L)	113.90 ± 4.44	178.68 ± 5.16	56.9	<0.05
	ALP (U/L)	21.21 ± 0.41	59.37 ± 1.04	179.9	<0.05
	Bilirubin (mg/dL)	0.33 ± 0.05	1.46 ± 0.12	341.1	<0.05
	Albumin (g/dL)	4.12 ± 0.06	3.90 ± 0.049	-5.4	<0.05
	T.protein (g/dL)	6.20 ± 0.03	5.72 ± 0.05	-7.6	<0.05
	Ammonia (µmol/L)	46.34 ± 0.95	91.31 ± 0.61	97.0	<0.05
	IL-1β (pg/ml)	3.19 ± 0.07	5.52 ± 0.17	73.1	<0.05
	IL-6 (pg/ml)	10.44 ± 0.37	19.91 ± 0.34	90.7	<0.05
	TNF-α (pg/ml)	0.34 ± 0.03	10.09 ± 0.35	94.5	<0.05
	S-100β (pg/ml)	26.33 ± 0.48	46.87 ± 0.36	78.0	<0.05
Liver	Ammonia (µmol/g wet tissue)	0.61 ± 0.01	6.06 ± 0.20	896.5	<0.05
	IL-1β (pg/mg tissue)	394.49 ± 37.04	705.88 ± 12.41	78.9	<0.05
	IL-6 (pg/mg tissue)	117.95 ± 5.75	204.65 ± 11.62	73.5	<0.05
	TNF-α (pg/mg tissue)	262.13 ± 5.76	398.15 ± 5.51	51.9	<0.05
Brain	Ammonia (µmol/g wet tissue)	0.34 ± 0.01	4.04 ± 0.15	1079.9	<0.05
	IL-1β (pg/mg tissue)	1097.58 ± 30.67	1607.70 ± 23.43	46.5	<0.05
	IL-6 (pg/mg tissue)	312.41 ± 5.11	444.64 ± 7.29	42.3	<0.05
	TNF-α (pg/mg tissue)	747.34 ± 9.77	1506.01 ± 15.50	101.5	<0.05

Several factors affect the rate of protein synthesis such as hormones (e.g thyronine and cortisol), nutritional status, environment and health. Albumin is synthesized in the parenchymal cells of the liver. It is responsible for maintaining the colloid oncotic pressure of plasma. In addition, it acts as a non-specific transport mechanism for a number of substances, including fatty acids, urates, calcium, bilirubin and various drugs.[30] In the current work, the levels of albumin and total protein recorded significant ($p<0.05$) decrease in hepatic encephalopathy rats after TAA administration as compared to control ones (Table 1). These results may be attributed to the increment of injury in the hepatocytes, the reduction in SH-protein bond production, the disturbance in the endocrine system (Growth hormone, thyroxin, triiodothyronine, cortisol and insulin) or/and the alterations in sequences of RNA. These data are in harmony with.[31]

Nitrogenous substances specifically ammonia are thought to be a critical component in the development of HE. In the presence of liver dysfunction, serum concentrations of ammonia

are elevated with impairment in urea synthesis and the brain acts as a major ammonia detoxification pathway. Hyperammonemia causes increment in the conversion of glutamine in astrocytes, which triggers a cascade of events. The elevated glutamine levels permit an osmotic gradient with resultant astrocyte swelling, which is believed to precipitate molecular events such as activation of inhibitory (γ-aminobutyric acid) and impairment of excitatory (glutamate, catecholamine) neurotransmitter systems, resulting in neural inhibition.[32]

In the current investigation, TAA-administrated rats showed significantly ($p<0.05$) elevation in the levels of serum ammonia associated with a remarkable increment in the concentrations of ammonia in both liver and brain tissues (Table 1). These results may be attributed to the disturbance in the urea cycle due to the deactivation of urea cycle enzymes such as; carbamyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), glutamine synthetase(GS), argininosuccinate synthetase (ASS), argininosuccinic acid lyase and arginase. These results seemed to be in complete accordance with studies made by.[24]

Ammonia may be important in the pathogenesis of HE, however, increasing evidences suggest that inflammation also plays a significant role in the pathogenesis of HE and brain edema.[33] It is characterized by the presence of proinflammatory cytokines and interferons which secrete from activated macrophages and Kuppfer cells and leading to hepatic necrosis.[34] Circulating cytokines may enter the brain in regions lacking a blood-brain barrier (BBB) or by activation of the endothelial vasculature, where receptors for these cytokines are localized. Circulating cytokines may also alter blood-brain barrier permeability, promoting increased brain uptake of toxins such as ammonia.[35&1]

Under normal physiological conditions, the healthy liver produces undetectable levels of cytokines IL-6.[36] Kupffer cells play an important role in the regulation of inflammatory processes in liver by secreting cytokines such as IL-6 and ROS which promote chemotaxis, phagocytosis and further ROS production. IL-6 is a cytokine involved not only in inflammation but also in the regulation of metabolic, regenerative and neural processes.[37] Increased expression IL-6 was observed in rats with TAA-induced acute hepatic failure and encephalopathy.[38] Serum levels of IL-1 β have been reported to be involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis.[39]

In the current study, TAA-administrated rats showed significantly ($p<0.05$) higher levels of the serum proinflammatory cytokine TNF-α in serum, liver and brain than those of their

corresponding control group (Table 1). This finding is compatible with the hypothesis that HE is associated with cerebral inflammation. **Sener et al. (2007)**[39] reported that tissue damages occurred in TAA-treated rats were accompanied by elevated serum levels of pro-inflammatory mediators (IL-1β, IL-6 and TNF-α). The authors suggested these changes due to increased production of inducible nitric oxide (iNOS) and activation of nuclear factor-kB (NF-kB). The possible mechanism that circulating proinflammatory cytokines may stimulate the activation of astrocytes in HE through circumventricular organs that lack a blood brain barrier to secrete cytokines.[40] As a result, the circulating inflammatory process would cause a worsening of brain inflammation and a release like a chute of proinflammatory cytokines. Therefore, cerebral inflammation actually may play a more significant role in the pathogenesis of brain edema in hepatic encephalopathy.

A correlation between serum S100β level and intracranial pressure has also been found in a porcine model of acute liver failure. These findings point to the determination of serum S100β as a potential noninvasive alternative to monitoring of intracranial pressure in patients with acute liver failure.[41] In patients with cirrhosis, serum S100β was reported to be specifically elevated in the presence of subclinical or early portal systemic encephalopathy.[42] In the current study, the serum levels of S100β were markedly higher in hepatic encephalopathy rats as compared with those of their corresponding control ones (Table 1).

Lactulose is mainly used in the treatment of constipation and hepatic encephalopathy. Their beneficial effects reflect their ability to reduce the intestinal production/absorption of ammonia.[43] In the current study, the hepatic encephalopathy rats which were treated with lactulose showed a significant ($p<0.01$) decrease in the liver functions (ALT, AST, ALP & bilirubin) and ammonia concentration associated with a significant amelioration occurred in the serum, liver & brain cytokines (IL-1β, IL-6 & TNF-α) levels through the whole experiment periods (1, 2, 4 & 8 wks) (Tables (2-5). These data may be attributable to the reduction in ammonia absorption by conversion of ammonia to the poorly absorbable ammonium ion and decrease in ammonia production in situ by bacteria. These results are in accordance with that evidence obtained by **Prasad et al. (2007)**[43] **and Mullen & Prakash (2012).**[44]

Lactulose exert a beneficial effect by many proposed ways: (1) A laxative effect: the colonic metabolism of the nonabsorbable disaccharides results an increase in intraluminal gas formation, an increase in intraluminal osmolality, a reduction in intraluminal pH, and an

overall decrease in transit time; (2) Bacterial uptake of ammonia: the intraluminal changes in pH result in a leaching of ammonia from the circulation into the colon. The colonic bacteria use the released volatile fatty acids as substrate and proliferate. In doing so, they use the trapped colonic ammonia as a nitrogen source for protein synthesis. (3) Reduction of intestinal ammonia production: nonabsorbable disaccharides inhibit glutaminase activity and interfere with the intestinal uptake of glutamine and its subsequent metabolism to ammonia. (4) Beneficial effects on the gut microbiome: cirrhosis is associated with dysbiosis and changes to the colonic mucosal microbiome.[45]

Table (2):- Effect of lactulose and/or taurine on liver function of hepatic encephalopathy rats (Mean±SE).

Parameters		Groups				
		Control	HE	HE + Lactulose	HE + Taurine	HE + Mix
ALT (U/L)	1 week	$30.05 \pm 0.44^{A}_{a}$	$69.25 \pm 1.21^{D}_{a}$	$64.71 \pm 0.67^{C}_{d}$	$67.89 \pm 0.53^{D}_{d}$	$61.83 \pm 0.46^{B}_{d}$
	2 weeks	$29.92 \pm 0.31^{A}_{a}$	$92.52 \pm 1.26^{E}_{b}$	$58.51 \pm 0.63^{C}_{c}$	$62.81 \pm 0.82^{D}_{c}$	$52.69 \pm 0.71^{B}_{c}$
	4 weeks	$29.73 \pm 0.31^{A}_{a}$	$109.80 \pm 2.58^{E}_{c}$	$48.58 \pm 0.60^{C}_{b}$	$53.57 \pm 0.75^{D}_{b}$	$41.70 \pm 0.56^{B}_{b}$
	8 weeks	$30.06 \pm 0.15^{A}_{a}$	$176.96 \pm 2.99^{D}_{d}$	$41.41 \pm 0.46^{B}_{a}$	$49.52 \pm 0.46^{C}_{a}$	$31.91 \pm 0.50^{A}_{a}$
AST (U/L)	1 week	$110.32 \pm 1.43^{A}_{a}$	$233.34 \pm 3.98^{D}_{a}$	$218.10 \pm 2.95^{C}_{d}$	$226.68 \pm 3.46^{D}_{d}$	$202.30 \pm 1.60^{B}_{d}$
	2 weeks	$110.68 \pm 0.39^{A}_{a}$	$278.56 \pm 3.81^{E}_{b}$	$200.02 \pm 1.52^{C}_{c}$	$209.30 \pm 1.08^{D}_{c}$	$175.98 \pm 2.71^{B}_{c}$
	4 weeks	$110.56 \pm 0.37^{A}_{a}$	$326.24 \pm 8.69^{E}_{c}$	$158.32 \pm 1.57^{C}_{b}$	$185.70 \pm 2.08^{D}_{b}$	$138.74 \pm 1.31^{B}_{b}$
	8 weeks	$109.62 \pm 0.29^{A}_{a}$	$475.7 \pm 10.61^{D}_{d}$	$138.32 \pm 1.30^{B}_{a}$	$159.38 \pm 1.2^{C}_{a}$	$118.18 \pm 1.42^{A}_{a}$
ALP (U/L)	1 week	$20.89 \pm 0.13^{A}_{a}$	$80.83 \pm 1.04^{D}_{a}$	$76.80 \pm 0.89^{C}_{d}$	$79.68 \pm 0.73^{D}_{d}$	$70.94 \pm 0.51^{B}_{d}$
	2 weeks	$20.52 \pm 0.32^{A}_{a}$	$93.84 \pm 1.65^{E}_{b}$	$71.27 \pm 0.61^{C}_{c}$	$75.51 \pm 0.33^{D}_{c}$	$57.11 \pm 1.14^{B}_{c}$
	4 weeks	$19.98 \pm 0.23^{A}_{a}$	$101.07 \pm 0.96^{E}_{c}$	$56.52 \pm 0.56^{C}_{b}$	$63.72 \pm 1.03^{D}_{b}$	$44.24 \pm 0.99^{B}_{b}$
	8 weeks	$19.99 \pm 0.17^{A}_{a}$	$149.71 \pm 4.26^{E}_{d}$	$44.03 \pm 0.79^{C}_{a}$	$51.65 \pm 0.70^{D}_{a}$	$28.92 \pm 1.04^{B}_{a}$
Bilirubin (mg/dL)	1 week	$0.36 \pm 0.012^{A}_{a}$	$1.91 \pm 0.02^{D}_{a}$	$1.79 \pm 0.01^{B}_{c}$	$1.86 \pm 0.01^{C}_{d}$	$1.74 \pm 0.02^{B}_{d}$
	2 weeks	$0.358 \pm 0.007^{A}_{a}$	$2.16 \pm 0.04^{E}_{b}$	$1.74 \pm 0.02^{C}_{c}$	$1.82 \pm 0.01^{D}_{c}$	$1.38 \pm 0.03^{B}_{c}$
	4 weeks	$0.35 \pm 0.006^{A}_{a}$	$2.45 \pm 0.03^{E}_{c}$	$1.40 \pm 0.02^{C}_{b}$	$1.63 \pm 0.02^{D}_{b}$	$0.94 \pm 0.02^{B}_{b}$
	8 weeks	$0.38 \pm 0.02^{A}_{a}$	$2.91 \pm 0.06^{E}_{d}$	$0.94 \pm 0.02^{C}_{a}$	$1.31 \pm 0.01^{D}_{a}$	$0.61 \pm 0.01^{B}_{a}$
Albumin (g/dL)	1 week	$4.124 \pm 0.018^{D}_{a}$	$3.61 \pm 0.03^{A}_{d}$	$3.72 \pm 0.02^{B}_{a}$	$3.60 \pm 0.02^{A}_{a}$	$3.81 \pm 0.02^{C}_{a}$
	2 weeks	$4.106 \pm 0.02^{E}_{a}$	$3.34 \pm 0.04^{A}_{c}$	$3.79 \pm 0.01^{C}_{b}$	$3.64 \pm 0.01^{B}_{b}$	$3.88 \pm 0.01^{D}_{b}$
	4 weeks	$4.114 \pm 0.02^{E}_{a}$	$3.21 \pm 0.02^{A}_{b}$	$3.92 \pm 0.02^{C}_{c}$	$3.74 \pm 0.02^{B}_{c}$	$3.98 \pm 0.01^{D}_{c}$
	8 weeks	$4.106 \pm 0.01^{D}_{a}$	$3.04 \pm 0.02^{A}_{a}$	$3.99 \pm 0.01^{C}_{d}$	$3.93 \pm 0.01^{B}_{d}$	$4.07 \pm 0.01^{D}_{d}$
T. protein (g/dL)	1 week	$6.192 \pm 0.02^{D}_{a}$	$5.47 \pm 0.02^{A}_{d}$	$5.62 \pm 0.02^{B}_{a}$	$5.47 \pm 0.02^{A}_{a}$	$5.80 \pm 0.02^{C}_{a}$
	2 weeks	$6.19 \pm 0.02^{E}_{a}$	$5.24 \pm 0.03^{A}_{c}$	$5.81 \pm 0.01^{C}_{b}$	$5.62 \pm 0.02^{B}_{b}$	$5.92 \pm 0.02^{D}_{b}$
	4 weeks	$6.186 \pm 0.01^{D}_{a}$	$5.08 \pm 0.02^{A}_{b}$	$5.94 \pm 0.02^{C}_{c}$	$5.76 \pm 0.02^{B}_{c}$	$5.99 \pm 0.01^{C}_{c}$
	8 weeks	$6.186 \pm 0.01^{E}_{a}$	$4.97 \pm 0.03^{A}_{a}$	$5.99 \pm 0.01^{C}_{c}$	$5.91 \pm 0.01^{B}_{d}$	$6.12 \pm 0.01^{D}_{d}$

- Means bearing different superscripts (A, B, C, D, E) within the same row are differ significantly (P<0.01).

- Means bearing different subscripts (a, b, c, d) within the same column are differ significantly (P<0.01).

Moreover, lactulose is not broken down by intestinal disaccharidases and thus reaches the colon, where bacteria will metabolize the sugar to acetic acid and lactic acid. The

acidification of the colon may underlie its cathartic effect. The passage of ammonia into the colonic lumen leads to its incorporation into bacteria with the resulting decrease of portal blood ammonia. As a result, peripheral levels of ammonia are reduced and the total body pool of urea decreases.[46&44]

Yu et al. (2015)[47] investigated the potential influence of lactulose administration on liver regeneration. They concluded that lactulose administration accelerates posthepatectomized liver regeneration in rats by inducing hydrogen, which may result from attenuation of the oxidative stress response and excessive inflammatory response. Also, in the mouse model of human ulcerative colitis, **Chen et al. (2013)**[25] found that lactulose can prevent the development of dextran sodium sulfate induced colitis and alleviate oxidative stress in the colon, probably by increasing endogenous H_2 production.

Table (3):- Effect of lactulose and/or taurine on serum ammonia, S100β and cytokines of hepatic encephalopathy rats (Mean±SE).

Parameters		Groups				
		Control	HE	HE + Lactulose	HE + Taurine	HE + Mix
Ammonia (µmol/L)	1 week	46.31 ± 0.29 $^{A}_{a}$	122.08 ± 1.20 $^{E}_{a}$	109.28 ± 0.89 $^{C}_{d}$	116.01 ± 1.66 $^{D}_{d}$	99.28 ± 0.60 $^{B}_{d}$
	2 weeks	45.82 ± 0.28 $^{A}_{a}$	159.43 ± 2.16 $^{E}_{b}$	98.26 ± 0.75 $^{C}_{c}$	104.70 ± 0.43 $^{D}_{c}$	84.42 ± 1.05 $^{B}_{c}$
	4 weeks	46.02 ± 0.25 $^{A}_{a}$	167.002 ± 2.95 $^{E}_{c}$	81.78 ± 0.58 $^{C}_{b}$	91.74 ± 0.72 $^{D}_{b}$	68.30 ± 0.97 $^{B}_{b}$
	8 weeks	45.77 ± 0.29 $^{A}_{a}$	204.19 ± 2.91 $^{E}_{d}$	67.15 ± 0.72 $^{C}_{a}$	77.47 ± 1.07 $^{D}_{a}$	53.24 ± 1.08 $^{B}_{a}$
S100β (pg/ml)	1 week	26.54 ± 0.45 $^{A}_{a}$	71.22 ± 0.48 $^{E}_{a}$	44.46 ± 0.19 $^{C}_{d}$	46.03 ± 0.07 $^{D}_{d}$	43.36 ± 0.22 $^{B}_{d}$
	2 weeks	25.93 ± 0.35 $^{A}_{a}$	75.08 ± 0.43 $^{E}_{b}$	42.17 ± 0.18 $^{C}_{c}$	44.63 ± 0.23 $^{D}_{c}$	39.52 ± 0.24 $^{B}_{c}$
	4 weeks	26.65 ± 0.25 $^{A}_{a}$	81.57 ± 0.24 $^{E}_{c}$	39.47 ± 0.16 $^{C}_{b}$	41.78 ± 0.10 $^{D}_{b}$	34.60 ± 0.27 $^{B}_{b}$
	8 weeks	26.75 ± 0.24 $^{A}_{a}$	94.31 ± 1.34 $^{E}_{d}$	31.42 ± 0.49 $^{C}_{a}$	38.10 ± 0.21 $^{D}_{a}$	28.96 ± 0.41 $^{B}_{a}$
IL-1β (pg/ml)	1 week	3.23 ± 0.03 $^{A}_{a}$	8.14 ± 0.20 $^{E}_{a}$	4.95 ± 0.07 $^{C}_{d}$	5.42 ± 0.03 $^{D}_{d}$	4.40 ± 0.05 $^{B}_{d}$
	2 weeks	3.21 ± 0.02 $^{A}_{a}$	9.04 ± 0.09 $^{E}_{b}$	4.41 ± 0.03 $^{C}_{c}$	4.89 ± 0.03 $^{D}_{c}$	4.07 ± 0.05 $^{B}_{c}$
	4 weeks	3.20 ± 0.02 $^{A}_{a}$	9.94 ± 0.09 $^{E}_{c}$	4.05 ± 0.06 $^{C}_{b}$	4.27 ± 0.04 $^{D}_{b}$	3.68 ± 0.03 $^{B}_{b}$
	8 weeks	3.206 ± 0.02 $^{A}_{a}$	13.47 ± 0.28 $^{C}_{d}$	3.47 ± 0.03 $^{A}_{a}$	4.05 ± 0.08 $^{B}_{a}$	3.22 ± 0.02 $^{A}_{a}$
IL-6 (pg/ml)	1 week	10.50 ± 0.09 $^{A}_{a}$	36.61 ± 0.72 $^{E}_{a}$	17.04 ± 0.07 $^{C}_{d}$	18.89 ± 0.09 $^{D}_{d}$	15.94 ± 0.06 $^{B}_{d}$
	2 weeks	10.52 ± 0.05 $^{A}_{a}$	40.20 ± 0.14 $^{E}_{b}$	16.00 ± 0.05 $^{C}_{c}$	17.07 ± 0.14 $^{D}_{c}$	13.94 ± 0.13 $^{B}_{c}$
	4 weeks	10.47 ± 0.04 $^{A}_{a}$	43.23 ± 0.20 $^{E}_{c}$	13.96 ± 0.08 $^{C}_{b}$	15.57 ± 0.11 $^{D}_{b}$	12.64 ± 0.12 $^{B}_{b}$
	8 weeks	10.46 ± 0.03 $^{A}_{a}$	47.25 ± 0.48 $^{D}_{d}$	11.58 ± 0.16 $^{B}_{a}$	13.67 ± 0.15 $^{C}_{a}$	10.45 ± 0.05 $^{A}_{a}$
TNF-α (pg/ml)	1 week	5.20 ± 0.009 $^{A}_{a}$	18.46 ± 0.69 $^{D}_{a}$	9.35 ± 0.04 $^{C}_{d}$	10.05 ± 0.06 $^{C}_{d}$	8.84 ± 0.067 $^{B}_{d}$
	2 weeks	5.19 ± 0.02 $^{A}_{a}$	21.42 ± 0.25 $^{E}_{b}$	8.68 ± 0.05 $^{C}_{c}$	9.37 ± 0.03 $^{D}_{c}$	8.26 ± 0.08 $^{B}_{c}$
	4 weeks	5.22 ± 0.02 $^{A}_{a}$	24.96 ± 0.13 $^{D}_{c}$	8.35 ± 0.06 $^{C}_{b}$	8.52 ± 0.03 $^{C}_{b}$	7.56 ± 0.08 $^{B}_{b}$
	8 weeks	5.21 ± 0.02 $^{A}_{a}$	28.33 ± 0.39 $^{D}_{d}$	6.51 ± 0.15 $^{B}_{a}$	8.22 ± 0.15 $^{C}_{a}$	5.80 ± 0.14 $^{A}_{a}$

- Means bearing different superscripts (A, B, C, D, E) within the same row are differ significantly (P<0.01).

- Means bearing different subscripts (a, b, c, d) within the same column are differ significantly (P<0.01).

Taurine is an amino acid found in lower amounts in the tissues of many animals including humans and has a number of physiological functions. It is a potent free radical scavenger that attenuates the damage caused by excessive mitochondrial oxidative stress.[48] Moreover, taurine gives rise to alterations in mitochondria of disrupted hepatocytes. It shows the stabilization of mitochondrial membrane, leading to the improvement of mitochondrial function.[49] Also, Hepatoprotective feature of taurine is attributed to its inhibitory activity on generation of ROS.[50] In the current work, administration of taurine not only effectively inhibited thioacetamide-induced hepatic encephalopathy, but also it has a great effect on serum, liver and brain ammonia as well as inflammatory cytokines (IL-1β, IL-6 & TNF-α) (Tables 2-5). Taurine might inhibit thioacetamide bioactivation through CYP2E1inhibition and consequently prevent hepatic injury.[51] These results are in parallel with **Das et al. (2010).**[14]

It has been found that elevated brain ammonia caused oxidative stress, bioenergetic failure, alterations in pH and Ca^{2+} homeostasis and electrophysiological disturbances in CNS.[52] The anti-oxidative, mitochondrial protecting and calcium sequestering effects of taurine might be involved in its protective properties against chronic and acute liver injury-induced hyperammonemia and its consequent brain injury.[53]

Table (4):- Effect of lactulose and/or taurine on ammonia and cytokines in liver tissue of hepatic encephalopathy rats (Mean±SE).

Parameters		Groups				
		Control	HE	HE + Lactulose	HE + Taurine	HE + Mix
Ammonia (µmol/g wet tissue)	1 week	0.598 ± 0.01 $^{A}_{a}$	8.99 ± 0.17 $^{E}_{a}$	5.62 ± 0.05 $^{C}_{d}$	8.35 ± 0.16 $^{D}_{d}$	4.57 ± 0.04 $^{B}_{d}$
	2 weeks	0.60 ± 0.01 $^{A}_{a}$	12.22 ± 0.15 $^{E}_{b}$	4.51 ± 0.04 $^{C}_{c}$	5.67 ± 0.05 $^{D}_{c}$	2.64 ± 0.07 $^{B}_{c}$
	4 weeks	0.596 ± 0.01 $^{A}_{a}$	15.46 ± 0.26 $^{E}_{c}$	2.64 ± 0.07 $^{C}_{b}$	4.53 ± 0.04 $^{D}_{b}$	1.46 ± 0.07 $^{B}_{b}$
	8 weeks	0.595 ± 0.02 $^{A}_{a}$	18.51 ± 0.36 $^{D}_{d}$	1.29 ± 0.06 $^{B}_{a}$	2.63 ± 0.05 $^{C}_{a}$	0.90 ± 0.01 $^{AB}_{a}$
IL-1β (pg/mg tissue)	1 week	402.96 ± 7.9 $^{A}_{a}$	1297.8 ± 7.4 $^{E}_{a}$	722.1 ± 6.01 $^{C}_{d}$	1271.6 ± 8.4 $^{D}_{d}$	644.34 ± 4.4 $^{B}_{d}$
	2 weeks	399.44 ± 4.6 $^{A}_{a}$	1631.4 ± 34.7 $^{E}_{b}$	638.3 ± 4.99 $^{C}_{c}$	727.5 ± 6.16 $^{D}_{c}$	563.5 ± 7.9 $^{B}_{c}$
	4 weeks	398.18 ± 3.1 $^{A}_{a}$	1910.4 ± 40.9 $^{D}_{c}$	564.3 ± 7.51 $^{B}_{b}$	660.00 ± 18.9 $^{C}_{b}$	427.1 ± 10.1 $^{A}_{b}$
	8 weeks	396.4 ± 1.8 $^{A}_{a}$	2516.8 ± 69.4 $^{C}_{d}$	407.74 ± 8.25 $^{A}_{a}$	556.40 ± 7.53 $^{B}_{a}$	405.1 ± 5.29 $^{A}_{a}$
IL-6 (pg/mg tissue)	1 week	115.48 ± 3.1 $^{A}_{a}$	351.2 ± 7.51 $^{D}_{a}$	213.26 ± 2.71 $^{C}_{d}$	354.80 ± 4.33 $^{D}_{d}$	188 ± 3.68 $^{B}_{d}$
	2 weeks	115.8 ± 1.78 $^{A}_{a}$	434.84 ± 13.78 $^{D}_{b}$	185.98 ± 3.77 $^{B}_{c}$	208.80 ± 3.13 $^{C}_{c}$	165.9 ± 4.88 $^{B}_{c}$
	4 weeks	117.4 ± 0.84 $^{A}_{a}$	514.36 ± 14.7 $^{E}_{c}$	164.5 ± 3.66 $^{C}_{b}$	188.04 ± 2.79 $^{D}_{b}$	142.4 ± 3.89 $^{B}_{b}$
	8 weeks	117.46 ± 0.6 $^{A}_{a}$	615.84 ± 12.6 $^{C}_{d}$	122.38 ± 4.95 $^{A}_{a}$	157.9 ± 3.35 $^{B}_{a}$	118.06 ± 0.8 $^{A}_{a}$
TNF-α (pg/mg tissue)	1 week	261.9 ± 3.05 $^{A}_{a}$	574.3 ± 8.26 $^{D}_{a}$	379.2 ± 3.55 $^{C}_{c}$	574.52 ± 3.21 $^{D}_{d}$	321.2 ± 4.10 $^{B}_{c}$
	2 weeks	262.88 ± 1.3 $^{A}_{a}$	761.9 ±17.47 $^{D}_{b}$	312.78 ± 4.5 $^{B}_{b}$	383.58 ± 4.15 $^{C}_{c}$	268.3 ± 2.48 $^{A}_{b}$
	4 weeks	261.3 ± 1.07 $^{A}_{a}$	921 ± 13.6 $^{C}_{c}$	254.84 ± 4.70 $^{A}_{a}$	318.0 ± 3.98 $^{B}_{b}$	250.5 ± 3.47 $^{A}_{a}$
	8 weeks	258.96 ± 1.3 $^{A}_{a}$	1064.5 ± 35.1 $^{B}_{d}$	254.5 ± 6.27 $^{A}_{a}$	256.7 ± 2.51 $^{A}_{a}$	245.0 ± 1.76 $^{A}_{a}$

- Means bearing different superscripts (A, B, C, D) within the same row are differ significantly (P<0.01).

- Means bearing different subscripts (a, b, c) within the same column are differ significantly (P<0.01).

One important function of taurine is its neuro-protective function. **El Edrissi & Trenkner (1999)**[54] reported that taurine can effectively prevent glutamate-induced neuronal injury in cultured neurons. In addition, they demonstrated that taurine can protect against H_2O_2-induced cell injury in PC12 cell cultures by reducing H_2O_2.[55] In addition, we have also demonstrated that glutamate-induced activation of calpain is inhibited by taurine resulting in decrease of formation of hetero-dimers of Bcl-2 and Bax and the subsequent release of cytochrome C and the apoptosis cascade.[56] The effect of taurine on brain astrocytes might be involved in the mechanism by which this amino acid prevents brain edema in *in vivo* conditions. **Cauli et al. (2014)**[57] mentioned that the predominant role of neuroinflammation rather than brain edema in the pathogenesis of CNS injury in HE. Taurine and its derivatives act as anti-inflammatory agents[58], one of the interesting mechanisms of neuroprotective properties of taurine against hyperammonemia might be mediated by this pathway.

Co-administration of lactulose and taurine to the hepatic encephalopathy rats provided a marked correction effects on all studied parameters (Table 2-5). The mixture brought the levels of all parameters closer to normal levels, than observed in lactulose or taurine alone. These results may be due to the synergistic effects of them to correct and repair the damage occurs in liver and brain of hepatic encephalopathy rats.

Table (5):- Effect of lactulose and/or taurine on ammonia and cytokines in brain tissue of hepatic encephalopathy rats (Mean±SE).

Parameters		Groups				
		Control	HE	HE + Lactulose	HE + Taurine	HE + Mix
Ammonia (µmol/g wet tissue)	1 week	$0.342 \pm 0.01^{A}_{a}$	$5.61 \pm 0.12^{D}_{a}$	$5.27 \pm 0.03^{C}_{d}$	$5.47 \pm 0.04^{C}_{c}$	$3.80 \pm 0.10^{B}_{d}$
	2 weeks	$0.34 \pm 0.003^{A}_{a}$	$5.85 \pm 0.14^{E}_{a}$	$3.68 \pm 0.06^{C}_{c}$	$5.22 \pm 0.06^{D}_{b}$	$3.22 \pm 0.07^{B}_{c}$
	4 weeks	$0.33 \pm 0.003^{A}_{a}$	$6.84 \pm 0.03^{E}_{b}$	$3.19 \pm 0.03^{C}_{b}$	$5.24 \pm 0.08^{D}_{b}$	$1.08 \pm 0.08^{B}_{b}$
	8 weeks	$0.344 \pm 0.01^{A}_{a}$	$7.96 \pm 0.18^{D}_{c}$	$2.25 \pm 0.06^{B}_{a}$	$3.67 \pm 0.06^{C}_{a}$	$0.52 \pm 0.02^{A}_{a}$
(pg/mg tissue)	1 week	$1096.00 \pm 15.9^{A}_{a}$	$1830.00 \pm 15.16^{E}_{a}$	$1578.60 \pm 9.35^{C}_{d}$	$1800.00 \pm 7.88^{D}_{d}$	$1423.00 \pm 20.81^{B}_{d}$

	2 weeks	1074.00± 4.18 $^{A}_{a}$	1913.00 ± 13.74 $^{E}_{b}$	1432.20 ± 7.67 $^{C}_{c}$	1729.60 ± 8.18 $^{D}_{c}$	1292.00 ± 11.47 $^{B}_{C}$
	4 weeks	1074.00 ± 2.68 $^{A}_{a}$	2504.00 ± 24.20 $^{E}_{c}$	1289.60 ± 8.48 $^{C}_{b}$	1582.00 ± 11.88 $^{D}_{b}$	1125.00 ± 12.54 $^{B}_{b}$
	8 weeks	1096.00 ± 16.1 $^{A}_{a}$	2946.00 ± 44.14 $^{B}_{d}$	1156.00 ± 26.27 $^{A}_{a}$	1433.00 ± 8.32 $^{C}_{a}$	1081.00 ± 4.69 $^{A}_{a}$
IL-6 (pg/mg tissue)	**1 week**	272.54 ± 41.0 $^{A}_{a}$	490.2 ± 6.27 $^{C}_{a}$	442.6 ± 2.62B$^{C}_{d}$	482.2 ± 4.92 $^{C}_{b}$	392.1 ± 6.54 $^{B}_{d}$
	2 weeks	314.66 ± 2.60 $^{A}_{a}$	544.36 ± 7.86 $^{D}_{b}$	383.90 ± 3.99 $^{B}_{c}$	424.1 ± 9.99 $^{C}_{a}$	372.02 ± 4.79 $^{B}_{c}$
	4 weeks	315.72 ± 2.54 $^{A}_{a}$	594.1 ± 7.07 $^{D}_{c}$	372± 3.37B$^{C}_{b}$	406.9 ± 25.56 $^{C}_{a}$	347.2 ± 7.9$^{B}_{b}$
	8 weeks	314.9 ± 4.82 $^{A}_{a}$	648.5 ± 6.41 $^{D}_{d}$	332.14 ± 3.91$^{B}_{a}$	383.52 ± 4.18 $^{C}_{a}$	326.10 ± 5.5A$^{B}_{a}$
TNF-α (pg/mg tissue)	**1 week**	745.9 ± 6.15 $^{A}_{a}$	1706.00 ± 15.05 $^{E}_{a}$	1490.60 ± 6.33 $^{C}_{c}$	1652.70 ± 6.50 $^{B}_{c}$	1152.00 ± 31.4$^{B}_{d}$
	2 weeks	755.9 ± 3.11 $^{A}_{a}$	1887.00 ± 10.12 $^{E}_{b}$	1143.00 ± 25.97 $^{C}_{b}$	1632.30± 6.15 $^{D}_{c}$	1056.00 ± 19.44 $^{B}_{c}$
	4 weeks	751.3 ± 2.47 $^{A}_{a}$	227.00 ± 30.73 $^{D}_{c}$	1231.00 ± 188.5 $^{B}_{b}$	1494.98 ± 8.76 $^{C}_{b}$	932.14 ± 12.6 $^{A}_{b}$
	8 weeks	745.8 ± 5.06 $^{A}_{a}$	2665.00 ± 33.33 $^{D}_{d}$	903.30± 22.78 $^{B}_{a}$	1148.70± 25.6 $^{C}_{a}$	774.08 ± 9.94 $^{A}_{a}$

- Means bearing different superscripts (A, B, C, D) within the same row are differ significantly (P<0.01).

- Means bearing different subscripts (a, b, c) within the same column are differ significantly (P<0.01).

Table (6):- Illustrate the Person's correlation between S-100β and different parameters.

Parameters		**S-100β (pg/ml)**	
Serum	**ALT (U/L)**	r	0.928*
	AST (U/L)	r	0.943*
	ALP (U/L)	r	0.925*
	Bilirubin (mg/dL)	r	0.895*
	Albumin (g/dL)	r	-0.946*
	T.protein (g/dL)	r	-0.954*
	Ammonia (µmol/L)	r	0.968*
	IL-1β (pg/ml)	r	0.948*
	IL-6 (pg/ml)	r	0.985*
	TNF-α (pg/ml)	r	0.987*
Liver	**Ammonia (µmol/g wet tissue)**	r	0.876*
	IL-1β (pg/mg tissue)	r	0.948*
	IL-6 (pg/mg tissue)	r	0.940*
	TNF-α (pg/mg tissue)	r	0.987*
Brain	**Ammonia (µmol/g wet tissue)**	r	0.975*
	IL-1β (pg/mg tissue)	r	0.956*
	IL-6 (pg/mg tissue)	r	0.959*
	TNF-α (pg/mg tissue)	r	0.942*

-(r): means correlation coefficient value.

-(*): means P value (p<0.001).

Furthermore, positive correlations were found between S100β and liver enzymes, bilirubin, ammonia and cytokines in all groups but negative correlations were found between S100β and total protein and albumin (Table 6). The elevation in the serum level of S100β in hepatic encephalopathy rats group may be attributed to the considerations of S100β which is dependent on the bases for two different ways, one in which the tumor and surrounding tissue actively produce and extravasate S100β (e.g.; glioblastoma) and another in which only adjacent normal tissue produces S100 in regions with normal BBB permeability (e.g. lymphoma). However, the blood-brain barrier disruption (BBBD) will cause the breakdown of this normal BBB and will lead to elevation in serum S100β levels.[59]

From the above cited data, it could be concluded that the administration of lactulose and/or taurine to HE rats group appeared to be safe and may be associated with a better outcome. These may be attributed to the pharmakientic and pharmadynamic properties of lactulose and taurine which acts as neuroprotection agents as well as hepatoprotective agents.

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