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ORIGINAL ARTICLE

Thioacetamide-induced acute hepatic encephalopathy: central vs peripheral effect of Allicin

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Abstract

Hepatic encephalopathy (HE) is a debilitating and life-threatening disease. Results from acute or chronic liver failure and is characterized by abnormal cerebral and neurological alterations. This study aimed at investigating the effect of allicin, the major functional component in freshly crushed garlic extract, on thioacetamide (TAA)-induced HE in rats. Induction of HE by a single dose of TAA (300 mg/kg; I.P.) was associated with a marked elevation in the serum levels of alanine aminotransferase, aspartate aminotransferase, bilirubin, albumin, total protein, blood urea nitrogen and serum ammonia besides reduction in the serum level of albumin. Moreover, it was accompanied with an increase in the hepatic and brain levels of inflammatory mediators; TNF-α and IL-1β as well as elevation of the hepatic and brain levels of oxidative stress biomarkers; reduced glutathione and lipid peroxidation evidenced by malondialdeyde. Oral administration of allicin (50, 100 and 200 mg/kg; P.O.) for 6 days prior to TAA injection restored the serum liver function, hepatic and brain levels of inflammatory mediators as well as oxidative stress biomarkers in a dose-dependent manner. From our results, it can be concluded that allicin has a protective effect on TAA-induced HE in rats in a dose-dependent manner due to its powerful antioxidant and anti-inflammatory properties.

Keywords Hepatic encephalopathy · Liver failure · Allicin · Antioxidant · Anti-inflammatory

Introduction

Hepatic encephalopathy (HE) is a decline of brain function which occurs when the liver is unable to remove toxins from the blood. It is a consequence of liver disease complicating up to 25% of presentations of acute liver failure (ALF) (Ahboucha and Butterworth 2004; Bernal et al. 2007). Clinical manifestations of HE include lethargy, depressed consciousness, loss of cognitive function and coma. Minimal hepatic encephalopathy affects about 60–80% of patients with liver cirrhosis (Ortiz et al. 2005).

Ammonia is generated by the degradation of amines, amino acids and urea by enteric bacteria. In liver failure, the ammonia produced in the gut bypasses the liver, and high amounts of it reach the systemic circulation. In addition, liver impairment reduces the conversion of ammonia into urea, resulting in further accumulation of ammonia in the blood (Afifi et al. 2020; Ahmed et al. 2014). Thus, ammonia plays an important role in the pathogenesis of HE (Cooper and Plum 1987). It is directly toxic to the brain, and in acute liver failure causes disturbance of inhibitory and excitatory neurotransmission. Ammonia impairs brain energy metabolism, alters expression of several genes that code for important proteins involved in brain function, and impairs auto-regulation of cerebral blood flow (Seyan et al. 2010; Larsen et al. 2000).

Infection and inflammation are leading factors in delirium development. Acute and chronic liver failure neurological manifestations are exacerbated by inflammation. In case of HE, inflammation can affect brain functions directly or indirectly and systemically (Bernal et al. 2007). Systemic inflammatory response syndrome (SIRS) is a phenotype of cytokine storm. SIRS plays an important role in exacerbating HE manifestations and development of covert HE into sever one in which the

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markers of inflammation correlates with neurodegenerative severity (Tranah et al. 2013).

The dysregulation of innate immune system in patients with liver failure makes them immune-suppressed and at high risk of infection development. Patients with ALF and cirrhosis have neutrophil abnormalities (Shawcross et al. 2010). Neutrophils swelling in case of hyper-ammonemia, develop impairment in their phagocytic activity and produce reactive oxygen species (Shawcross et al. 2008). Impaired neutrophil phagocytic activity correlates with the peak arterial concentration of ammonia (Taylor et al. 2013). Hyperammonemia, SIRS, and dysregulation of innate immune system play a great role in pathogenesis of HE (Tranah et al. 2013).

In this study acute HE was induced by TAA as a series of experimental models found that HE model could be established successfully in rats via administration of single dose of 300 mg/kg TAA (Mladenović et al. 2014). TAA-induced HE by intraperitoneal injection has high similarity to the HE phenotypes in human, good reproducibility, high success rate, and easy operation (Felipo 2013).

Garlic (*Allium sativum*) has been known as an important therapeutic agent many years ago. From freshly crushed garlic, allicin is produced from its allin (S-allylcysteinesulfoxide) precursor using alliinase enzyme as a catalyst (Kendler 1987). Properties of garlic are attributed to allicin (2-pro-pene-1sulfinothioic acid S-2-propenyl ester) which is the major functional component in freshly crushed garlic extract. Allicin is an organosulfur pungent smelling compound with anti-oxidant and anti-inflammatory properties. Compounds that have antioxidant properties may act against free radical directly by scavenging ROS. On the other hand, antioxidants may act indirectly by increasing the activity of endogenous cellular antioxidant defenses (Kelsey et al. 2010). In addition, allicin can affect endogenous immune system (Borlinghaus et al. 2014). The migration of neutrophilic granulocytes into epithelia is a crucial process during inflammation that is inhibited by allicin. The release of TNFα-dependent pro-inflammatory cytokines in intestinal epithelia is inhibited by allicin (Hobauer et al. 2000).

This study aimed to investigate the effect of allicin, the major functional component in freshly crushed garlic extract, on on the liver and brain damage associated with HE induced by thioacetamide (TAA) in rats.

Materials and methods

Animals

Thirty adult male albino rats; weighing about 120-150 g, were purchased from the Vacsera, Giza, Egypt. Rats were randomly divided into five groups (6 rats/group) in standard polypropylene cages in the animal house of October University of Modern Sciences and Arts (MSA). Rats were kept in standard environmental condition of temperature about 25 °C with regular 12 h light/12 h dark cycle and allowed access to commercially available rat pellet diet and water. These conditions were maintained until the end of the experiment. Rats are left a week for acclimatization before the beginning of the study.

Drugs and chemicals

Allicin was purchased from boots pharmacies in United States and thioacetamide (TAA) was purchased from Sigma–Aldrich. Any other chemicals used were of analytical grades.

Induction of hepatic encephalopathy

Hepatic encephalopathy was induced with a single intraperitoneal injection of TAA (300 mg/kg; I.P.) (Mladenović et al. 2014). Rats were injected with TAA dissolved in sterile distilled water to a concentration of 300 mg/kg after weighing them.

Study design

Evaluation of the protective efficacy of allicin administration against TAA-induced acute HE

In the current study, HE was induced by single I.P. injection of TAA (300 mg/kg). Rats were randomly divided into five experimental groups (6 rats/group). Group I served as negative control group which received saline only. Group II served as positive control which received I.P single dose of TAA 300 mg/kg. Groups III, IV and V received oral allicin at doses 50, 100, 200 mg/kg; respectively, for 6 consecutive days prior to TAA injection. Forty-eight hours after the TAA injection, rats were sacrificed, blood samples were collected and the serum was separated. Liver and brain were isolated, each divided into two parts; one part used for preparation of liver and brain homogenates and the other part stored in formalin for histopathological examination.

Measurement of biochemical parameters

Measurement of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin, total protein, blood urea nitrogen (BUN) and serum ammonia

All serum liver biomarkers were determined using reagent kits (Biodiagnostic, Dokki, Giza, Egypt). Serum levels of (ALT) and (AST) were determined following the methods of Lippes et al (Lippes et al. 1972). The absorbance was measured at 546 nm and the results were expressed as U/L. Bilirubin levels

were determined according to the method of Walter and Gerade (1970). The absorbance was measured at 546 nm and the results are expressed in milligrams per decilitre of serum. Albumin levels were determined following the method of Doumas et al. (1971). The absorbance was measured at 630 nm and the results are expressed in milligrams per decilitre of serum. Total protein levels were measured according to the method of Peterson (1983). The absorbance was measured at 535 nm and the results are expressed in milligrams per decilitre of serum. Blood urea nitrogen (BUN) levels were measured following the methods of Tiffany et al. (1972). Serum ammonia levels were measured according to the methods of Jensen et al. (1998).

Preparation of liver and brain homogenates and estimation of oxidative stress biomarkers and inflammatory mediators

The livers and brains homogenization was carried out and homogenate was used for the estimation of liver and brain contents of malondialdehyde (MDA), reduced glutathione (GSH), tumer necrosis factor alpha (TNF-α), and interlukin-1beta (IL-1β). MDA levels were determined using the method of Matsumiya and Hoshino (2003). GSH levels were measured following the method of Vaziri et al. (2000). The tissue level of tumor necrosis factor alpha (TNF-α) was determined with an enzyme-linked immunosorbent assay (ELISA) using a test reagent kit (Raybiotech) according to the method of Sun et al. (2004). The tissue levels of interleukin (IL-1β) were also determined with ELISA using a test reagent kit (ImmunoBiological Laboratories) according to the method of Govindan and DeVita (2009).

Histopathology

Autopsy samples were taken from the liver and brain of rats in different groups and fixed in 10% formalin for twenty-four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embeded in paraffin at 56 degrees in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μm thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin &eosin stain for routine examination through the light electric microscope (Banchroft et al. 1996).

The main histopathological criteria used for assessment was illustrated in Table 1. The total pathologic score is the sum of the individual score of each histopathological parameter. For assessment of astrocytes size, the mean diameter of astrocytes was measured in random fifteen high power field (HPF) per group (Ishak et al. 1995).

Statistical analysis

Data are presented as mean value ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. *: significantly different from control group at $P \leq 0.05$. @: significantly different from TAA induced HE group at $P \leq 0.05$.

Results

The effect of allicin on serum liver functions in TAA-induced HE in rats

Induction of HE by a single injection of TAA (300 mg/kg; i.p) showed an elevation in the ALT, AST, bilirubin 19%, 33%, 18%; respectively and a suppression in albumin and total protein levels by 30% and 31%; respectively. Pre-treatment of TAA-injected rats with allicin (50 mg/kg; P.O.) for 6 days showed a decrease in AST, ALT and bilirubin by 8%, 8% and 2%; respectively and increase in albumin and total protein by 7%, and 2%; respectively. Pre-treatment of TAA-injected rats with allicin (100 mg/kg; P.O.) for 6 days showed a decrease in AST, ALT and bilirubin by 11%, 12% and 12%; respectively and increase in albumin and total protein by 12% and 6%; respectively. Pre-treatment of TAA-injected rats with allicin (200 mg/kg; P.O.) for 6 days showed a decrease in AST, ALT and bilirubin by 14%, 30% and 13%; respectively and increase in albumin and total protein by 16% and.17%; respectively (Table 2).

Table 1 The main histopathological criteria

Grade	Cellular balloononing	Apoptosis	Portal Inflammation	Focal Necrosis
0	Absent	None	None	Absent
1	Mild (focal, some hepatic lobules)	Sparse	Mild (some or all portal areas)	One focus/LPF(10X)
2	Moderate (focal,<50% of hepatic lobules	Few	Moderate (some or all portal areas)	Tow to 4foci/LPF(10X)
3	Severe (diffuse, >50 of hepatic lobules	Numerous	Marked (marked in all portal areas)	≥5foci/LPF(10X)

The effect of allicin on blood urea nitrogen, serum and brain ammonia level in TAA-induced HE in rats

Induction of HE by a single injection of TAA (300 mg/kg; I.P.) showed an elevation in BUN, serum and brain ammonia level by 41%, 39% and 142%; respectively. Pre-treatment of TAA-injected rats with allicin (50 mg/kg; P.O.) for 6 days showed a decrease in BUN, serum and brain ammonia level by 5%, 10% and 47%; respectively. Pre-treatment of TAA-injected rats with allicin (100 mg/kg; P.O.) for 6 days showed a decrease in BUN, serum and brain ammonia level by 31%, 12% and 79%; respectively. Pre-treatment of TAA-injected rats with allicin (200 mg/kg; P.O.) for 6 days showed a decrease in BUN, serum and brain ammonia level by 32%, 24% and 119%; respectively (Table 3).

The effect of allicin on hepatic and brain oxidative stress biomarkers in TAA-induced HE in rats

Induction of HE by a single injection of TAA (300 mg/kg; I.P.) showed an elevation in liver MDA by 58% and suppression in hepatic glutathione by 26%. Pre-treatment of TAA-injected rats with allicin (50, 100, 200 mg/kg; P.O.) for 6 days showed a decrease in liver MDA level by 18% and 29% and 21%; respectively and an increase in hepatic GSH level by 28%, 34% and 40%; respectively (Table 4).

On the other hand, induction of HE by a single injection of TAA (300 mg/kg; I.P.) showed an elevation in brain MDA by 73% and suppression in brain glutathione by 47%. Pre-treatment of TAA-injected rats with allicin (50, 100, 200 mg/kg; P.O.) for 6 days showed a decrease in brain MDA level by 35%, 30% and 44%; respectively and an increase in brain GSH level by 29%, 7% and 36%; respectively (Table 4).

The effect of allicin on hepatic and brain inflammatory mediators in TAA-induced HE in rats

Induction of HE by a single injection of TAA (300 mg/kg; I.P.) showed an elevation in hepatic TNF-α and IL-1β levels by 111% and 50% while brain TNF-α and IL-1β by 147% and 110%; respectively. Pre-treatment of TAA-injected rats with allicin (50 mg/kg; P.O.) for 6 days showed a decrease in hepatic TNF-α and IL-1β levels 22% and 11% levels and brain TNF-α and IL-1β levels by 15% and 35%; respectively. Pre-treatment of TAA-injected rats with allicin (100 mg/kg; P.O.) for 6 days showed a decrease in hepatic TNF-α and IL-1β levels by 27% and 18% and brain TNF-α and IL-1β levels by 37%, and 39%; respectively. Pre-treatment of TAA-injected rats with allicin (200 mg/kg; P.O.) for 6 days showed a decrease in hepatic TNF-α and IL-1β levels by 34% and 23% and brain TNF-α and IL-1β levels by 51%, and 49%; respectively (Fig. 1).

The effect of allicin on liver and brain histopathological alterations in TAA-induced HE in rats

Liver isolated from TAA-induced HE rats showed inflammatory cells infiltration with few fibroblastic cells proliferation originated in the portal area and extended between the fatty degenerated hepatocytes in the parenchyma. Karyomegalocyte with prominent nucleoli were detected in some of the hepatocytes with inflammatory cells infiltration in between. The portal area showed congestion in the portal vein with periductal fibrosis surrounding the dilated bile ducts (Figs. 2b, c). Similarly, brain tissue showed nuclear pyknosis and degeneration and cellular edema were detected in the astrocytes of the cerebral cortex. There was focal gliosis in the cerebral cortex. The striatum

Table 2 The effect of allicin on serum liver functions in TAA-induced HE in rats

Parameter Groups	Alanine aminotransferase ALT (U/L)	Aspartate transaminase AST(U/L)	Bilirubin BIL (mg/dl)	Albumin (mg/dl)	Total protein (mg/dl)
Normal control (saline)	31.59±0.46	40.02±1.09	0.87±0.01	3.99±0.15	6.92±0.25
TAA-induced HE (300 mg/kg; I.P.)	37.56*±0.69	53.37*±2.26	1.02*±0.02	2.79*±0.07	4.76*±0.05
TAA+Allicin (50 mg/kg; P.O.)	35.07*@±0.28	50.29*±0.611	1.01*±0.01	3.05*±0.14	4.88*±0.095
TAA+Allicin (100 mg/kg; P.O.)	34.19*@±0.61	48.76*@±0.36	0.923*@±0.007	3.28*±0.19	5.13*±0.13
TAA+Allicin (200 mg/kg; P.O.)	33.35@±0.42	41.57@±0.62	0.916*@±0.007	3.44*@±0.04	5.91*@±0.04

HE was induced in rats with a single injection in TAA 300 mg/kg i.p. TAA injected rats where pretreated with allicin (50, 100, and 200 mg\kg P.O.) for 6 days. Forty-eight hours after TAA injection, blood was collected, centrifuged and sera was used for ALT, AST, bilirubin, albumin, and total protein measurement. Data are presented as mean value ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. *: significantly different from control group at P≤0.05.@: significantly different from TAA induced HE group at P≤0.05

Table 3 The effect of allicin on BUN, serum and brain ammonia in TAA induced HE in rats

Parameter	Blood urea nitrogen (BUN) (mg/dl)	Serum ammonia (nmol/L)	Brain ammonia (nmol/L)
Normal control (saline)	18.37±0.43	8.19±0.16	2.24±0.07
TAA induced HE (300 mg/kg)	25.86*±0.94	11.41*±0.17	5.42*± 0.11
TAA+Allicin (50 mg/kg)	24.96*±0.63	10.38*@±0.04	4.37*@±0.04
TAA+Allicin (100 mg/kg)	20.09@±0.56	10.54*@±0.06	3.65*@±0.06
TAA+Allicin (200 mg/kg)	19.93@±0.74	9.50*@±0.04	2.77*@±0.06

HE was induced in rats with a single injection in TAA 300 mg/kg i.p. TAA injected rats where pretreated with allicin (50, 100, and 200 mg\kg P.O.) for 6 days. Forty-eight hours after TAA injection, blood was collected, centrifuged and sera was used for BUN and ammonia measurement. Rats were sacrificed. Brain was isolated, brain tissue was homogenized and homogenate was used for brain ammonia measurement. Data are presented as mean value ± SEM and as % change from normal control group. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons.

*: significantly different from control group at $P \leq 0.05$

showed edema in the perivascular tissue surrounding the blood vessels (Figs. 3c, d).

On the other hand, the liver isolated rats protected by allicin (50 mg/kg) showed inflammatory cells infiltration with few fibrosis were detected in the portal area. The portal area of rats protected by allicin (50 mg/kg; P.O.) showed few inflammatory cells infiltration associated with diffuse degeneration in the hepatocytes. Liver isolated from allicin (100 mg/kg; P.O.) group had few inflammatory cells infiltration was detected in the portal area (Table 5, Figs. 2d-i).

Moreover, the cerebral cortex of rats treated with allicin (50 mg/kg) showed nuclear pyknosis and degeneration in the astrocytes with perivascular edema. However, the cerebral cortex of rats protected with allicin (100 and 200 mg/kg) showed intact histological structure, while the striatum showed diffuse as well as mild perivascular edema (Table 5, Fig. 3e, j).

Discussion

Liver damage can be induced via administration of several agents such as carbon, D-galactose amine, acetaminophen and TAA. In the current study, acute liver injury and HE were induced through single I.P. injection of TAA (300 mg/kg). The current study showed a significant elevation in BUN, serum and brain ammonia level in TAA treated rats which indicate liver injury. In accordance with Grant et al. (2018) our results showed that marked elevation in ALT, AST and bilirubin level with decrease in albumin level.

In case of liver injury, there is a state of hyperammonemia. Ammonia bypass liver into systemic circulation and then astrocytes in the brain take ammonia and convert glutamate into glutamine. Astrocytic swelling and cerebral edema occurs due to osmotic effect of glutamine **(**Butterworth et al. 2009**)**. In brain, astrocytes are the main sites that reveal the histopathological findings as removal of ammonia from brain depends mainly on glutathione synthetase which is localized in astrocytes **(**Butterworth et al. 2009**)**. In agreement, our histopathological examination results of brain tissue showed nuclear pyknosis, degeneration and cellular edema in the astrocytes of the cerebral cortex in TAA-treated rats.

Rapid progression in severity of HE was observed in patients with ALF who had more marked inflammation (Tranah et al. 2013**)**. According to Bone et al. (2009) after liver injury,

Table 4 The effect of allicin on hepatic and brain MDA and GSH in TAA-induced HE in rats

Parameter Groups	Liver malondialdhyde (MDA) (nmol/ml)	Brain malondialdhyde MDA (nmol/ml)	Liver glutathione (GSH) (nmol/dl)	Brain glutathione (GSH) (nmol/dl)
Normal control (saline)	0.67±0.02	0.38±0.04	0.49±0.001	1.176±0.04
TAA- induced HE (300 mg/kg)	1.07*±0.02	0.67*±0.05	0.35*±0.01	0.63*±0.09
TAA+Allicin (50 mg/kg)	0.94*@±0.02	0.56*±0.01	0.45*±0.001	0.78*±0.01
TAA+Aliicin (100 mg/kg)	0.92*@±0.04	0.53*@±0.01	0.47*@±0.002	0.96*@±0.03
TAA+Allicin (200 mg/kg)	0.86*@±0.01	0.49@±0.01	0.49*@±0.002	1.04@±0.01

HE was induced in rats with a single injection of TAA 300 mg/kg i.p. TAA injected rats where pretreated with allicin (50, 100, and 200 mg\kg P.O.) for 6 days. Forty-eight hours after TAA injection, rats were sacrificed, liver and brain were isolated, liver and brain tissue were homogenized and homogenate was used for MDA and GSH measurement. Data are presented as mean value ± SEM and as % change from normal control group. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. *: significantly different from control group at $P \leq 0.05$; @: significantly different from TAA induced HE group at $P \leq 0.05$.

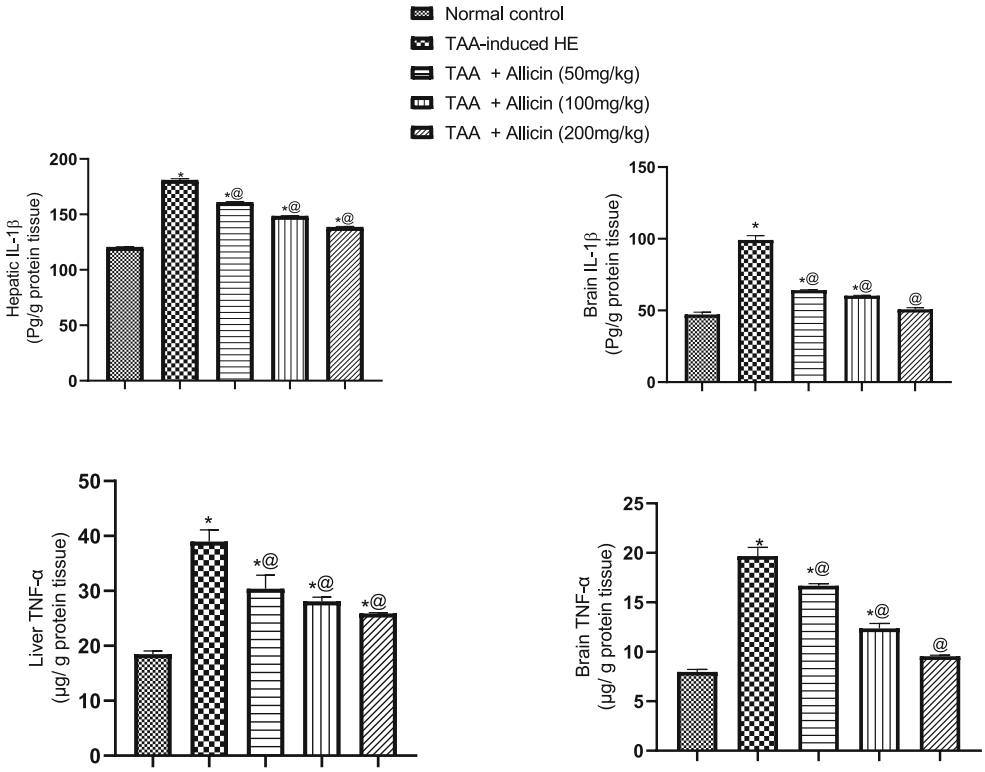

Fig. 1 The effect of allicin on liver (**a**), brain (**b**) IL-1β, liver (**c**) and brain (**d**) TNF-α levels in TAA-induced HE in rats. Normal control group received saline for 6 days, group 2 received TAA (300 mg/kg) single dose I.P, then euthanized after 48 h. group 3 received allicin (50 mg/kg orally) for 6 days followed by TAA administration then euthanized after 48 h, group 4 received allicin (100 mg/kg) orally for 6 days followed by TAA administration then euthanized after 48 h, group 5 received allicin (200 mg/kg orally) for 6 days followed by TAA administration then euthanized after 48 h. Rats were euthanized; liver and brain were isolated, then liver and brain homogenates were used for determination o TNF-α and IL-1β level. Data are presented as % change from normal control group. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. *: significantly different from control group at $P \leq 0.05$. @: significantly different from TAA induced HE group at $P \leq 0.05$

systemic inflammation develops. As a result, blood ammonia level is increased and pro-inflammatory mediators are released into the circulation. Results of our study showed an elevation in inflammatory mediators; TNF-α and IL-1β in both liver and brain tissue of TAA treated rats. In HE inflammation may develop directly within brain or indirectly affect the brain function. Astrocytic, microglial and neuronal dysfunction are the consequences of inflammation that arise directly within the brain (Wright and Jalan 2007). In accordance with Tranah et al. (2013) there is a synergistic relationship between ammonia and systemic inflammation in the pathogenesis of HE.

In accordance with the study done by Shawcross et al. (2008) which demonstrated that elevated ammonia blood levels in ammonia-fed rats and cirrhotic patients results in impairment of neutrophiles phagocytic activity as well as spontaneous release of ROS by neutrophiles and induction of oxidative stress. Our results in the current study showed marked elevation in hepatic and brain levels of MDA and reduction in GSH antioxidant enzyme. This significant increase in oxidative stress may indicate negative shift in the oxidant/antioxidant balance. The role of oxidative stress in pathogenesis of HE is proven by a study done by Afifi et al. (2020) which indicate that development of brain edema in dimethyl malate-induced oxidative stress in hyper ammonaemic portacaval anastomosis rats implies a synergistic effect between ammonia and oxidative stress.

Allicin is the main active ingredient in freshly crashed garlic. It has been reported to have anti-inflammatory and antioxidant properties (Shang et al. 2019; Rabinkov et al. 1998). In the current study, allicin was administrated orally in three different dose levels (50, 100, 200 mg/kg) for 6 days prior to TAA injection. Our results showed marked reduction in serum liver functions, ALT, AST, albumin, BUN and both serum and brain ammonia in a dose dependent manner when compared to TAA-induced HE group. Restoring serum liver functions and Lowering in BUN, and ammonia levels indicate the improvement in the ability of the liver to remove toxins; thus, these results imply the hepato-protective effect of allicin in HE. In addition, allicin has anti-oxidant properties. The antioxidant effect of allicin may be direct through scavenging of ROS or indirect by activating and increasing activity of endogenous cellular antioxidant defenses (Kelsey et al. 2010). In the current study, our results indicated that rats in groups that

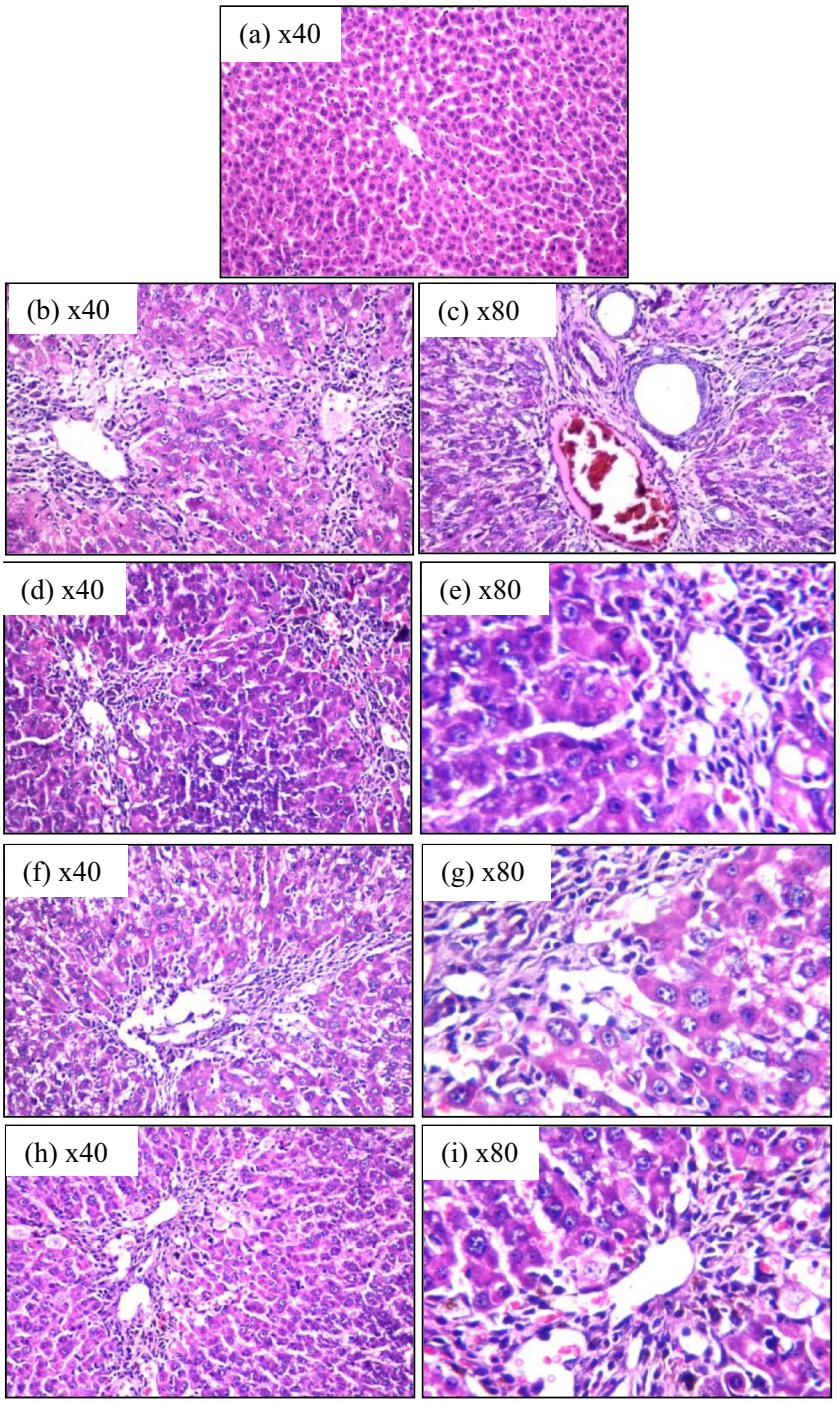

Fig. 2 The effect of allicin on liver histopathological alteration in TAA-induced HE in rats. **a** Liver of rat in group1 Showing normal histological structure of the central vein and surrounding hepatocytes in parenchyma (X40). **b** Liver of rat in group 2 Showing degenerative fatty change in the cytoplasm of hepatocytes with inflammatory cells infiltration and few fibroblastic cells proliferation, extending from the portal area to hepatic parenchyma between the degenerated hepatocytes (X40). **c** Liver of rat in group 2 Showing magnification to identify the congestion in portal vein with dilated bile duct and priductal fibrosis (X80). **d** Liver of rat in-group 3 showing inflammatory cells infiltration and few fibroblastic cells proliferation in the portal area (X40). **e** Liver of rat in group (3) showing inflammatory cells infiltration in portal area (X80). **f** Liver of rat in group (4) Showing few inflammatory cells with the degeneration in hepatocytes (X40). **g** Liver of rat in group (4) showing the magnification of (fig. b) to identify the inflammatory cells (X80). **h** Liver of rat in group (5) Showing few inflammatory cells infiltration in portal area (X40). **i** Liver of rat in group (5) showing the magnification of (fig.d) to identify the inflammatory cells infiltration in portal area (X80)

received allicin (50, 100 and 200 mg/kg) for 6 days prior to TAA developed marked reduction in both hepatic and brain MDA and significant increase in hepatic and brain GSH antioxidant enzyme in a dose dependent manner. These results may account for the anti-oxidant effect of allicin. According to Malhotra et al. (2008) synergistic relationship between hyperammonemia and systemic oxidative stress is responsible for development of brain edema in HE; therefore, when prooxidant enzymes inhibited, ROS are suppressed and HE is improved. Thus, reduction in MDA and elevation in GSH levels, in the present study, account for the powerful antioxidant effect of allicin in improving TAA-induced HE in rats.

In addition to the anti-oxidant properties, allicin has anti-inflammatory properties. Previous studies indicate that allicin can modulate the activity of inflammatory cells and inhibit the activation of NF-κB induced by TNF-α. Moreover, allicin can affect endogenous immune system, inhibit migration of

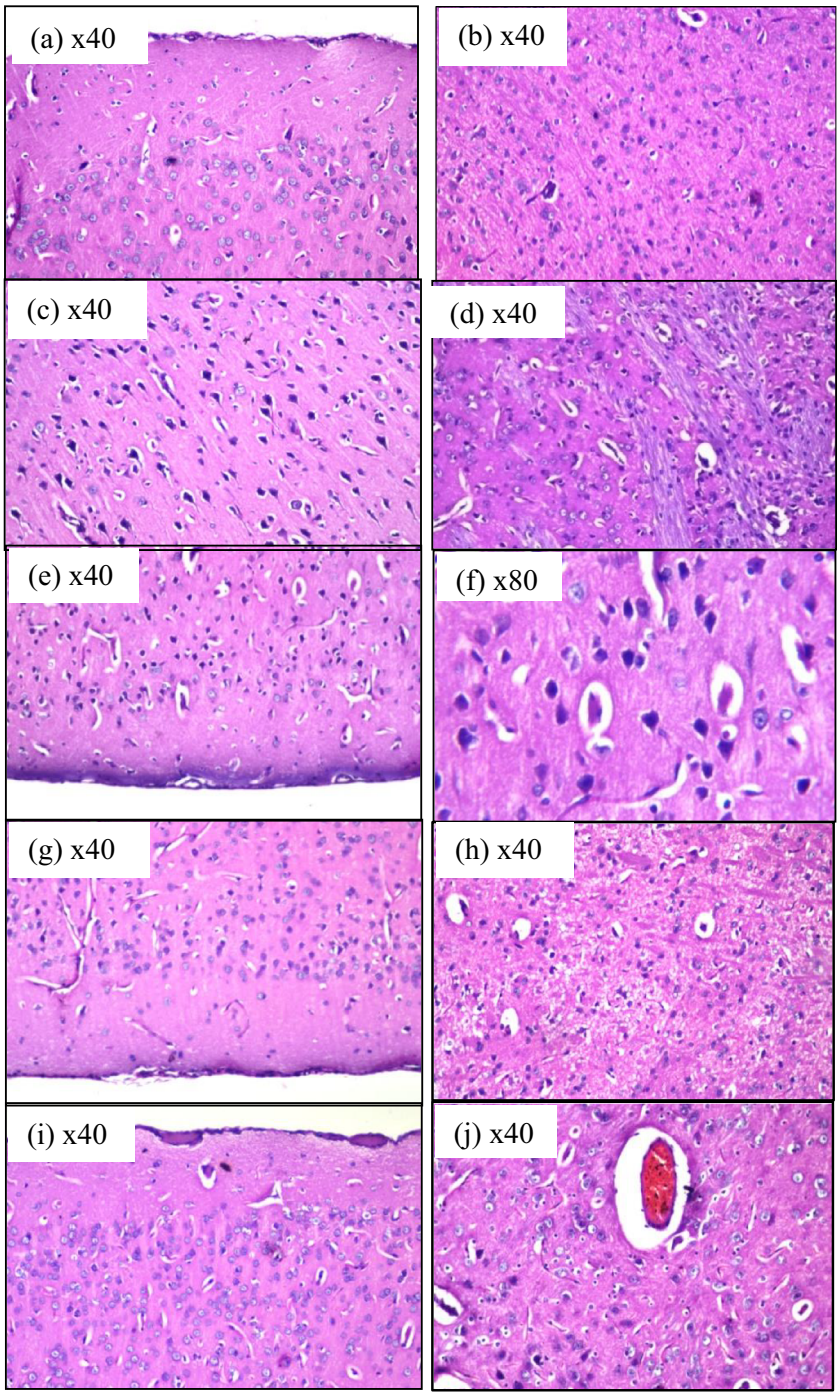

Fig. 3 The effect of allicin on brain histopathological alteration in TAA-induced HE in rats. **a** Brain of rat in group 1 Showing normal histological structure of cerebral cortex (X40). b Brain of rat in group 1 showing normal histological structure of striatum (X40). **c** Brain of rat of group 2 showing nuclear pyknosis with cellular edema in astrocytes of cerebral cortex (X80). [d] Brain of rat in group 2 Showing perivascular edema surrounding the blood capillaries of striatum (X40). **e** Brain of rats in group (3) showing nuclear pyknosis of the astrocytes with perivascular edema surrounding the blood vessles in cerebral cortex (X40). **f** Brain of rats in group (3) showing nuclear pyknosis of astrocytes and privascular edema in cerebral cortex (X80). **g** Brain of rat in group (4) showing normal histological structure of the cerebral cortex (X40). **h** Brain of rat in group (4) Showing edema in stria with perivascular edema surrounding the blood vessels (X40). **i** Brain of rat in group (5) Showing normal histological structure in cerebral cortex (X40). **j** Brain of rat in group (5) Showing congestion and perivascular oedema of blood vessels in striatum (X40)

neutrophilic granulocytes into epithelia, and inhibit release of TNF-α pro-inflammatory cytokines (Hobauer et al. 2000). These outcomes were emphasized by the results obtained from the present study, rats in groups that receive allicin (50, 100, 200 mg/kg) for 6 days prior to TAA showed a significant reduction in hepatic and brain levels of IL-1β and TNF-α in a dose dependent manner.

Anti-inflammatory effects of allicin in the current study are in agreement with Wang et al. (2017) and Bruck et al. (2005) who stated that allicin inhibits aminotransferase and TNF-α in mice pre-treated with allicin before concavalin A injection which induces liver injury. Significant reduction in hepatic and brain levels of IL-1β and TNF-α, in the current study, indicate that allicin is powerful anti-inflammatory. All results of biomarkers obtained in our study are supported by the results of histopathological examination of both liver and brain tissues of rats in groups that receive (50, 100, 200 mg/kg) for 6 days prior to TAA. These results of histopathological examination also indicate that allicin improve TAA-induced HE in a dose dependent manner.

Table 5 The effect of allicin on hepatic pathologic score and brain astrocyte size TAA-induced HE in rats

Parameter Groups	Total pathologic score	Mean astrocyte size (diameter in μm/HPF)
Normal control (saline)	0.32±0.1	6.64±0.41
TAA- induced HE (300 mg/kg)	8.80*±0.46	10.83*±0.33
TAA+Allicin (50 mg/kg)	0.52$^{@}$±0.23	7.88$^{*@}$±0.33
TAA+Allicin (100 mg/kg)	1.73$^{*@}$±0.19	7.89$^{*@}$±0.33
TAA+Allicin (200 mg/kg)	0.43$^{@}$±0.18	7.23$^{@}$±0.35

Conclusion

The current study showed that allicin has the ability to improve HE not only through its antioxidant and its anti-inflammatory effect but also it leads to reduction in serum and brain ammonia level in a dose dependent manner. In conclusion, ammonia plays an important role in the pathogenesis of HE but the poor correlation between its level and the severity of HE assure that other factors such as oxidative stress and inflammation are crucial factors in the pathogenesis of HE. Thus, agents with antioxidant and anti-inflammatory properties such as allicin are considered good candidates for treatment of HE.

Our study showed that allicin is powerful antioxidant and anti- inflammatory and it has a protective effect on TAA-induced HE in rats in a dose dependant manner. Allicin acts peripherally and centrally. Furthermore, its effect on brain is greater than its hepatic effects, which may be attributed to its ability to pass the blood brain barrier. Allicin showed significant reduction in the elevated ammonia levels in both serum and brain in a dose dependant manner. Thus, allicin plays a crucial role in the three main factors included in the pathogenesis of HE as it has a significant effect on liver enzymes, oxidative stress biomarkers, and inflammatory mediators, as well as ammonia content. In addition, from our study we can conclude that allicin can be used alone or as adjuvant therapy in management of HE.

@: significantly different from TAA induced HE group at $P \leq 0.05$

HE was induced in rats with a single injection of TAA 300 mg/kg i.p. TAA injected rats where pretreated with allicin (50, 100, and 200 mg\kg P.O.) for 6 days. 24 hours after the last dose of the drugs, mean total hepatic pathologic score and astrocyte size were evaluated. Results are expressed as mean ±SEM (n=8). *Significant difference from normal control group $p < 0.05$. @ Significant difference from TAA- injected group.

Author's contribution D. O. Saleh has designed the work, collected and interpreted the data, drafted and revised the article and has been responsible for the publication. D. F. Mansour has collected the data and drafted the article. A. M. Fayez has interpreted the data and finally approved the version for publishing.

Data availability The data that support the findings of this study are available from the corresponding author, D. Saleh, upon reasonable request.

Declarations

Conflict of interest Authors declare no conflict of interest. The study was carried out according to the guidelines of the Ethics Committee, Faculty of Pharmacy, October University for Modern Sciences and Arts (approval no. PH2/EC2/2016F).

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