Biochemical markers to the protective effects of Fructus Piperis Longi extract on Hepatic encephalopathy in rats

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A B S T R A C T

The present study was designed to elucidate the protective effect of subsequent pretreatment with Fructus Piperis Longi extract in liver and brain tissue of experimental rats exposed to Hepatic encephalopathy induced by intraperitoneal administration of TAA. Plf extract (50 mg/kg) was force-fed to rats every day, 7 days before administration of Thioacetamide. Thioacetamide 200 mg/kg was intraperitoneally administered to rats twice per week for four weeks. Administration of Plfext was extended during Thioacetamide. After 4 weeks, animals were sacrificed. Biochemical parameters in serum and homogenized liver and brain were tested. A significant increase in serum transglutaminase, alkaline phosphatase and gamma glutamyl transferase activity and plasma ammonia, a significant decrease in serum albumin and total protein was recorded in the liver of Thioacetamide-treated rats. Significant increases in liver and brain lipid peroxides associated to significant decreases of liver and brain reduced glutathione concentration and catalase activities were also recorded. Administration of Plfext treatment reduced the severity of liver fibrosis and oxidative damage which was substantiated by improvement of liver function detected by a decrease in serum aspartate amino transaminase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase activities and increase in total protein and Albumin. It was concluded that treatment of the ethanolic extract of Fructus Piperis Longi enhance Liver and brain parameter and enhanced antioxidant activities of liver and brain.

Keywords: Hepatic encephalopathy, TNF-α, MPO, COX2, Cholinesterase, β amyloid peptide.

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1. INTRODUCTION

Hepatic encephalopathy (HE) is a frequent complication and one of the most debilitating manifestations of liver disease severely affecting the lives of patients and their caregivers. Furthermore, cognitive impairment associated with cirrhosis results in utilization of more health care resources in adults than other manifestations of liver disease (Rakoski, et al., 2012).

HE is a brain dysfunction caused by liver insufficiency and/or porto-systemic shunting (PSS); it manifests as a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma (Ferenci, et al., 1998, Cordoba et al., 2011). It is based on the concept that encephalopathies are ‘‘diffuse disturbances of brain function (Cordoba et al., 2011) and that the adjective ‘‘hepatic’ implies a causal connection to liver insufficiency and/or perihepatic vascular shunting (Conn, et al., 1993).

The pathogenesis of this condition is not well defined. Accumulation of ammonia from the gut and other sources due to impaired hepatic clearance or porto-systemic shunting can lead to accumulation of glutamine in brain astrocytes, leading to swelling, which can be aggravated by hyponatremia. Other

mediators, such as benzodiazepine-like agonists, inflammatory cytokines, manganese, and neurosteroids, may play a role (Agrawal et al., 2012).

Thioacetamide, a thiono-sulfur containing compound (CH3CSNH2), undergoes an extensive metabolism to produce acetamide (CH3CONH2) and thioacetamide-Soxide (TAA-S-oxide) (Chieli et al., 1984) TAA-S-oxide thus produced is further metabolized, at least in part, by cytochrome P-450 monooxygenases. The subsequent product formed is TAA-S-dioxide, which exerts hepatotoxicity by binding to hepatocyte macromolecules and causes centrilobular necrosis by generation of reactive oxygen species (ROS) (Torres, et al., 1998, Ledda-Columbano et al., 1991, Bruck et al., 2002, Edmund et al., 2004 and Bruck et al., 2006). The produced ROS cause a variety of patho-physiological conditions by enhancing lipid peroxidation in bio-membranes. It subsequently can cause structural and functional degeneration of different enzymes and DNA inside the cell.

Plants have been the source of medicines since thousands of years. Members of the botanical family piperaceae were among the first cultivated plant and the species of the genus Piper are the important medicinal plants used in various systems of medicine (Kirtikar K.R. et al., 1933). Black pepper (Piper nigrum) and long pepper (Piper longum) are the best known spices in the family and are probably among the most recognized spices in the world. Both the peppers have been used medicinally for centuries. Black pepper alone accounts for about 35% of the world's total spice trade (Anonymous et al., 1998 and Bisht et al., 1963). Piper longum (Piperaceae), a slender aromatic climber, is a native of the Indo-Malayan region and grows wild in the tropical rain forests of India. The extract of the crude drug "Piperis Longi Fructus," the fruits of P. longum, is frequently used in folk medicine to treat bronchial trouble and is used as a carminative and analgesic (Parmar et al., 1997, Jung et al., 1989). Piperine was the first amide isolated from Piper species and was reported to display central nervous system depression, antipyretic, and anti-inflammatory activity (Lee et al., 1984, Woo et al., 1979). In the present work, we have investigated the hepato-protective activity of Fructus Piperis Longi against liver fibrosis and brain dysfunction.

2. MATERIAL AND METHODS

2.1. Chemical and antioxidant

Fructus Piperis Longi was obtained from the local market. Thioacetamide (purity~99%) (LobaChemi. Co, Delhi. India) was purchased from El-Gomhouria Co. for trading chemicals, Medicines and Medical Appliances, Egypt. All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

2.2. Instrument

Electric digital balance (Shimadzu, Type Ay 220, Japan), pH meter (Jenway, UK), homogenizer (Glas-col, TERRE HAUT, USA), cooling centrifuge (Memmert, Model K23, Germany), centrifuge (Janetzki, Model T30, Germany), shaker incubator (Lab-Line Instruments, USA) and spectrophotometer (Helios, UV/Visible, UK).

2.3. Experimental animals

All animal treatment procedures conformed to the National Institutes of Health (NIH) guidelines. (El Borai, M.S. et al., 2005).80 male albino rats (170-220 g) were used in this study. Animals were obtained from the National Centre for Radiation Research and Technology (NCCRT), Cairo, Egypt. The animals were housed in cages and maintained under standard conditions of ventilation, temperature and humidity. Animals received standard food pellets and water ad libitum.

2.4. Preparation and administration of dosage

Hepatic encephalopathy was induced by intraperitoneal administration of 200 mg/kg TAA twice per week for four weeks according to (El Borai et al., 2005).
Preparation of Fructus Piperis Longi extract, the ethanolic extract of Fructus Piperis Longi was prepared according to Christina et al. (Christina et al., 2006). Fructus Piperis Longi was obtained from the local market and was dried and powdered. About 500 g of dry powder was extracted with 5L of ethanol at 60-70°C for 72 hours by continuous hot percolation with a Soxhlet apparatus. The ethanolic extract was then filtered and concentrated by vacuum distillation to dry. The yield for 500g was 37 g. This dried extract was then stored at 4°C until use. Rats were force-fed 50 mg/kg of distilled water per day for five weeks starting from seven days before TAA administration.

2.5. Experimental design

The experimental animals were divided into four groups (n = 10), namely (1) Control: healthy animals received distilled water; (2) Plfext: animals received Fructus Piperis Longi extract; (3) TAA: animals were injected with TAA; (4) Plfext + TAA: animals received Fructus Piperis Longi extract and were injected with TAA.

2.6. Sampling

2.6.1. Blood

Blood samples were collected by heart puncture 1ml of the blood was collected on EDTA for ammonia analysis. The rest of blood samples were collected in dry, clean test tubes and allowed to clot for 30 min and serum was separated by centrifugation at 3000 rpm for 15. The serum was separated by automatic pipette and received in dry sterile tubes, processed directly for ALT, AST, ALP, GGT, Albumin and Total Protein. Then liver samples were collected for estimation of MDA, CAT, GSH, MPO, TNF, Cycloxygnase and IL6. And brain sample were collected for estimation of MDA, CAT, GSH, B-amyloid, NO and acetylcholinesterase.

2.6.2. Tissue Sample

The liver and brain tissue were rapidly dissected and excised, rinsed in saline solution for histopathological investigation.

2.7. Statistical analysis

The SPSS (version 20) was used in data analysis. Data were analyzed with one-way analysis of variance (ANOVA) followed by a post hoc test (LSD alpha) for multiple comparisons. The data were expressed as mean ± standard deviation (SD). P values < 0.05 were considered to be statistically significant.

3. RESULTS

Administration of Fructus Piperis Longi ethanol extract (Plfext) to rats, by force feeding, for a period of five weeks, did not show significant changes in all the studied parameters, indicating that the extract did not affect the liver and brain functions as shown in table (1, 2, 3). TAA administration causes a significant increase in serum (ALT, AST, ALP and GGT) and Plasma ammonia and a significant decrease in serum ALB and T. Protein, significant increase in Liver MDA, MPO, TNF, Cycloxygnase and IL6 and a significant decrease in Liver GSH and CAT and cause a significant increase in brain MDA, B-amyloid, NO and acetylcholinesterase and a significant decrease in brain GSH and CAT as shown in table (1, 2, 3). Administration of Fructus Piperis Longi ethanol extract (Plfext) to rats treatment exhibited a significant decrease in serum (ALT, AST, ALP and GGT) and Plasma ammonia, While a significant increase in serum ALB and T. Protein significant decrease in Liver MDA, MPO, TNF, Cycloxygnase and IL6 and a significant increase in Liver GSH and CAT and cause a significant decrease in brain MDA, B-amyloid, NO and acetylcholinesterase and significant increase in brain GSH and CAT as shown in table (1, 2, 3) compared with TAA group.

Table1. Liver functions of rats under different treatment conditions.

Treatment group	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	ALB (g/dl)	T. Protein (g/dl)	Plasma Ammonia (µg/dl)
Control (C)	46.65 ± 3.11 [b]	75.67 ± 3.34 [b]	105.83 ± 5.52 [b]	3.22 ± 0.29 [b]	3.87 ± 0.04 [b]	6.21± 0.10 [b]	440.8± 15.23 [b]
PLF ext	46.47 ± 2.05 [b]	72.33 ± 2.22 [b]	108.00 ± 3.56 [b]	2.89 ± 0.38 [b]	3.64 ± 0.12 [b]	6.09 ± 0.10 [b]	410.00 ± 4.4 [b]
TAA	155.33 ± 6.55 [a]	297.50 ± 11.68 [a]	149.58± 4.07 [a]	7.07± 0.40 [a]	2.75 ± 0.04 [a]	5.66 ± 0.03 [a]	801.83± 26.02 [a]
PLF ext+ TAA	[ab]65.35 ± 4.70	112.83 ± 4.30 [ab]	117.10± 2.69 [b]	4.23 ± 0.11 [b]	3.21 ± 0.03 [ab]	6.01 ± 0.05 [b]	554.00± 10.67 [ab]

Data are presented as (Mean ± S.E).S.E = Standard error. a: Significant difference from Control group at P≤ 0.05. b: Significant difference from TAA groupat *P*≤ 0.05.

Table 2: Catalase(CAT) , Reduced Glutathione (GSH), Lipid peroxides (LP), IL6,TNF, COX2 and MPO in liver tissue homogenates of rats under different treatment conditions.

Treatment group	CAT (U/L)	GSH (mg/gm tissue)	MDA (nmol/gm tissue)	IL6 (pg/mgptn)	TNF (pg/mgptn)	COX2	MPO (u/mgptn)
Control (C)	71.23± 0.32 [b]	171.61± 0.39[b]	108.34 ±9.03 [b]	32.7± 2.45 [b]	31.4± 1.53 [b]	1.0± 0.01 [b]	0.22± 0.01 [b]
PLF ext	69.73 ±0.87 [b]	169.75 ± 0.96[b]	134.17 ± 10.84 [b]	32.5± 1.8 [b]	30.3± 2.4 [b]	1.0± 0.01 [b]	0.24± 0.02 [b]
TAA	63.83 ±1.81 [a]	159.17 ± 1.14[a]	322.17 ± 12.03 [a]	144.6± 9.2 [a]	137.2± 6.9 [q]	11.2± 0.6 [a]	2.6± 0.18 [a]
PLF ext + TAA	68.91 ±0.62 [b]	170.42 ± 0.45[b]	182.50 ± 11.48[ab]	92.3± 3.6[ab]	94.1± 4.42[ab]	6.9± 0.03[ab]	0.94± 0.04 [b]

ata are presented as (Mean ± S.E). S.E = Standard error. a: Significant difference from Control group at P≤ 0.05. b: Significant difference from TAA groupat *P*≤ 0.05.

Table 3: Catalase(CAT), Reduced Glutathione (GSH), Lipid peroxides (LP), Cholinesterase, B-Amyloid and Nitric oxide (NO) in Brain tissue homogenates of rats under different treatment conditions.

Treatment group	CAT (U/L)	GSH (mg/gm tissue)	MDA (nmol/gm tissue)	Cholinest-erase (U/ml tissue)	B-Amyloid	NO
Control (C)	100.07 ± 0.58 [b]	184.86 ±1.31 [b]	88.37± 2.32 [b]	19.1± 0.63 [b]	1.04± 0.08 [b]	0.19± 0.01 [b]
PLF ext	97.43 ± 1.60 [b]	185.88 ± 1.83 [b]	84.93± 3.81 [b]	19.8± 1.5 [b]	1.10± 0.05 [b]	0.19± 0.01 [b]
TAA	89.93 ± 0.60 [a]	163.95 ± 0.32 [a]	202.90± 1.89 [a]	70.1± 4.7 [a]	6.00± 0.37 [a]	1.80± 0.05 [a]
PLF ext+ TAA	96.60 ± 1.39 [b]	176.00 ± 1.83 [ab]	133.27± 3.39 [ab]	34.6± 3.13[ab]	3.44± 0.29 [b]	0.33± 0.02[ab]

Data are presented as (Mean ± S.E). S.E = Standard error. a: Significant difference from Control group at *P*≤ 0.05. b: Significant difference from TAA groupat *P*≤ 0.05.

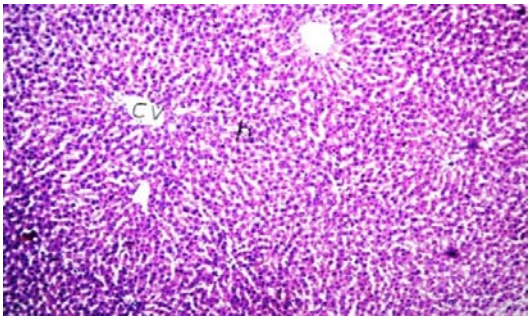

Fig (1): Liver of rat in gp (1) showing normal histological structure of central vein (cv) and surrounding hepatocytes (h) in the paranchyma. H&E ×16

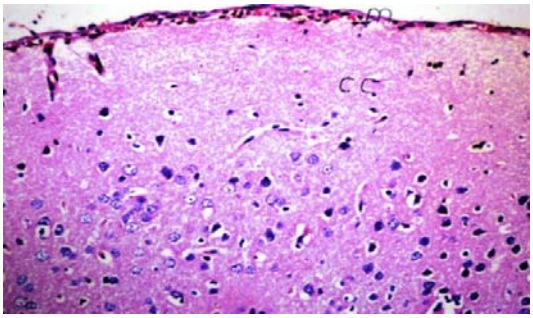

Fig (2): Brain of rat in gp (1) showing normal histological structure of the meninges(m) and cerebral cortex (cc). H&E ×40

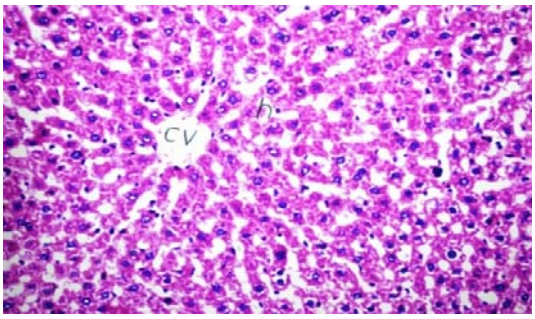

Fig (3): Liver of rat in gp (2) showing normal histological structure H&E ×40

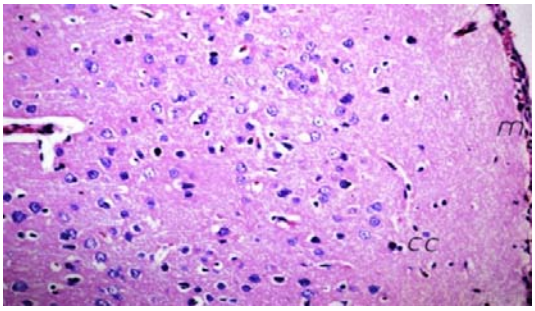

Fig (4): Brain of rat in gp (2) showing normal histological structure H&E ×40

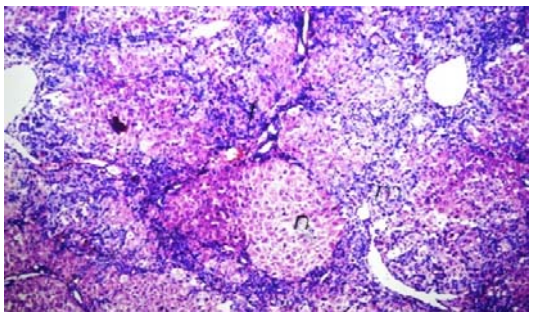

Fig (5): Liver of rat in gp (5) showing fibrosis (f) with inflammatory cells infiltration (m) dividing the degenerated dysplastic hepatocytes (n) in to lobules. H&E ×40

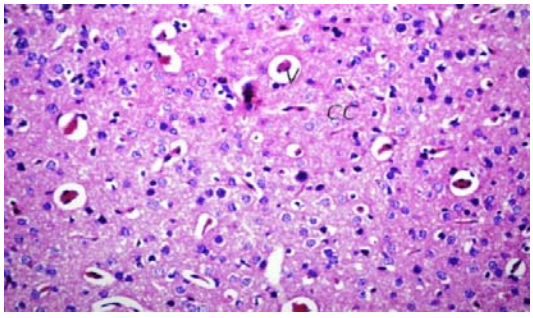

Fig (6): Brain of rat in gp (5) showing the congestion in capillaries (v) of cerebral cortex (cc). H&E ×40

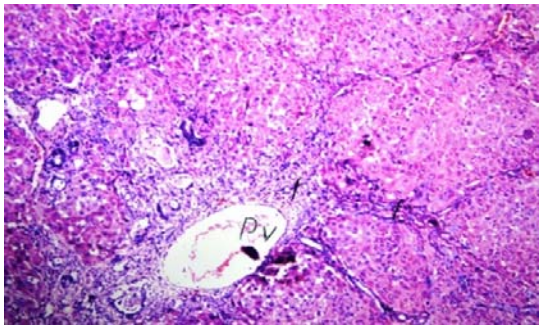

Fig (7): Liver of rat in gp (6) showing fibrosis(f) with inflammatory cells infiltration (m). H&E ×16

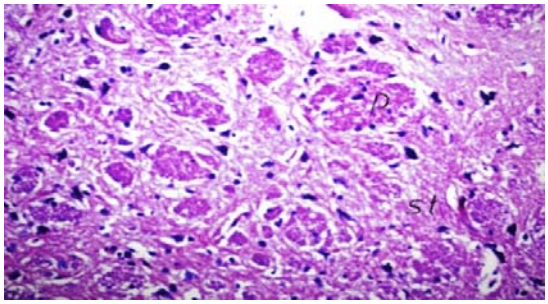

Fig (8): Brain of rat in gp (6) showing focal eosinophilic plagues formation (p) in striatum. H&E ×40

Table 4: The severity of Histopathological alterations in Hepatic and Brain tissues of different experimental groups

organ		C	PLF +C	TAA	PLF + TAA
Liver	Fibrosis dividing the degenerated paranchyma in to lobules	-	-	+++	++
Brain	Neuron degeneration &pyknosis in hippocampus	-	-	++	-
	Eaosinophilic plagues in straitum	-	-	++	+

(+++) Severe ;(100-75 %), (++) Moderate ;(75-50 %), (+) Mild ;(25-50 %), (-) Nil ;(0-25 %)

4. DISCUSSION

Liver plays the main role in metabolism of different nutrients, such as carbohydrates, proteins, and lipids; in addition, it shares in clearance of waste products resulting from metabolism and elimination of exogenous drugs and other xenobioc (Saleem et al., 2010).

Increase level of ammonia as result from liver failure is the main etiology of hepatic encephalopathy. Hyperammonemia affect the Function of mitochondria, which may result in reduction of ATP synthesis and it enhance free radicals production (Bachmann et al., 2002).

The most numerous findings of HE was psycho-motor deficiency in the animals. It has been indicated that the cholinergic system is associated with specific behavioral responses and cognitive processes both in healthy subjects and those with neurological dysfunction (Bartus et al., 1982).

Garcia-Ayllón et al. (2008) suggested that the impairment of the brain cholinergic system induced by liver disease might have a role in learning and memory functions. We found that thioacetamide caused serious neurological problems in the rats. Behavioral and psychiatric deficits are some of earliest signs of HE. We demonstrated that thioacetamide induced HE and low behavioral and motor scores in comparison with control groups.

TAA is considered as a selective hepatotoxin, which is used experimentally to induce hepatic failure. The result of TAA metabolism is highly reactive compound which is thioacetamide S dioxide; it binds to the tissue macromolecules and may result in hepatic necrosis (Reddy et al., 2004). In this study TAA treatment is accompanied by liver affection which is manifested by increased serum levels of liver enzymes (AST, ALT, ALP and GGT), increase in plasma ammonia and decrease in serum levels of Albumin and total proteins.

In addition, oxidative stress is manifested in hepatotoxic rats by increased MDA, MPO, TNF, Cycloxygnase and IL6 levels in the homogenate of liver tissues and there are diminution of GSH and CAT levels in liver and brain tissues homogenate and increase in brain MDA, B-amyloid, NO and acetylcholinesterase. Consequently, our study emphasizes that existence of liver failure and brain oxidative stress due to TAA administration, which is in agreement with (Bruck et al., 2004).

In accordance with our study, Mustafa, et al (2013) reported that TAA intoxication models registered significantly lower plasma total protein levels compared to healthy models. Moreover, Túnez et al. (2005) reported that TAA reduced the antioxidant status and enhanced lipid peroxidation in liver and brain tissues. Bastway et al. (2010) had confirmed the occurrence of oxidative stress through significant increase of MDA levels and significant decrease of GSH and NO in liver rats as an effect for TAA administration. Aydın et al. (2010) reported that TAA administration resulted in significant increases in plasma transaminase activities as well as hepatic hydroxyproline and lipid peroxide levels, while liver glutathione, superoxide-dismutase and glutathione

peroxidase protein expressions and activities decreased.

In a study for Fadillioglu et al. (2010) had studied the different oxidant and antioxidant parameters on three different parts of brain tissue in thioacetamide-induced HE in this rat model.

Then they reported that after thioacetamide injection, there was significant increase in the blood ammonia level and significant increase in ALT and AST activities in TAA administered group compared to the control group. In addition they reported a significant decrease in antioxidant enzyme activities in the cerebral cortex, brain stem and cerebellum. So they reported those protein oxidation and lipid peroxidations were produced in three brain tissue parts.

In a study of Ersin et al., (2010), after injection of TAA, there was a significant increase in blood ammonia level and significant increase in ALT and AST.

In accordance with the previous studies there are two major factors for induction of HE which are oxidative damage and increase blood ammonia level.

So to prevent HE you have to prevent ROS injury and decreasing blood ammonia level.

The nervous system is largely susceptible to oxidative damage because the brain is enriched with polyunsaturated fatty acids (Öztürk et al., 2008).

The nervous system also has low level of defense repair mechanisms and very high oxygen consumption against oxidative injurynisms and very high oxygen consumption against oxidative injury (Halliwell et al., 1985).

Our study in agreement, with different studies showed that the administration of PLF extract decrease the severity of hepatic encephalopathy owing to TAA intoxication, indicating that free radicals and reactive oxygen species have an important role in the pathogenesis of hepatic encephalopathy (Velvizhi et al., 2002) Najmi et al., (2010), SomayaZakaria et al., (2009).

In the present study, malonaldehyde (MAD) contents of hepatic and brain tissues and nitric oxide (NO) of brain tissue were significantly increased in rats adminstrated with TAA as compared to the control rats. On the other side, the liver and brain reduced glutathione (GSH) content and catalase (CAT) activity were decreased significantly in TAA rats. TAA-induced hepatotoxicity as a result of increased oxidative stress [Reactive oxygen species (ROS) and reactive nitrogen species (RNS)] due to a significant elevation in TNF-α and IL-6 in liver tissue and remarkable decrease in the activity of CAT and reduced glutathione (GSH) in both liver and brain of rats. These data are parallel to those obtained in the current investigation and confirmed by Murthy et al., (2001) and Tunez et al., (2005) demonstrated that TAA significant reduction in the activity of CAT in association with significant elevation in the levels of lipid peroxidation (TBARS and MDA) in both liver and brain of rats.

In addition to its important physiologic functions, nitric oxide is involved in various pathologic processes that lead to cytotoxicity (Hogg and Kalyanaraman 1999). Clear participation of NO in the pathogenic process of HE was reported in animal models (Bustamante et al., 2007). Because NO facilitates the systemic hypotension, some believe that by so doing NO will augment hepatic hypo perfusion and hepatocyte hypoxic damage. Chen et al., (2006) reported that there is a significant production of H2O2 in TAA-induced toxicity. Several reports indicated that exposure of cells to H2O2 promotes e NOS expression and thus NO synthesis [Drummond, et al., 2000].The interaction of nitric oxide (NO) with reactive oxygen species (ROS), especially superoxide anion, leads to the generation of highly reactive and cytotoxic byproducts, peroxynitrite, which can react with DNA, lipids and proteins (Beckman et al., 1996). For instance, peroxynitrite reacts with free tyrosine and tyrosine residues in protein molecules to produce nitrotyrosine. Alternatively, ROS can activate tyrosine to form tyrosyl, a radical that, in turn, oxidizes

NO to produce nitrotyrosine (Halliwell, 1997).

Histopathological investigation of brain tissue sections in TAA rats group showed congestion in the blood vessels of the meninges and cerebral cortex with diffuse gliosis in the cerebrum. Sever haemorrhage in the mininges covering the cerebellum associated with congestion in the blood vessels of the hippocampus and focal gliosis in the cerebrum. Haemorrhage in the fissure between the inferior cerebrums was also noticed. These findings are in accordance with those demonstrated by Jayakumar et al., (2011) who explained these results by the cytotoxic edema (astrocyte swelling) caused by Thioacetamide. Moreover, cerebral edema and associated increase in intracranial pressure due to TAA administration are believed to be the main cause death of patients with hepatic encephalopathy. These results are in accordance with those of Sathyasaikumar et al., Photomicrographs of brain tissue sections of rats in TAA-challenged group after 3 months revealed focal gliosis in the cerebrum associated with focal haemorrhages in the medulla oblongata. The current data come in line with those of Zimmermann et al., (1989) who observed that the nuclei of glial and neurnal cells in the brains of rats with HE were slightly swollen with no histological signs of brain edema.

In the present study, rats with higher levels of TNF- α, suggesting TNF- α may participate in the pathogenesis of hepatic encephalopathy. Till now, a number of potential mechanisms have been proposed to account the effect of TNF- α on the development of hepatic encephalopathy. TNF-α may cause cytotoxic effects on the cerebral endo the lial cells. Endo the lialdam age subsequently alters the blood-brain barrier permeability, leading to brain edema and death (Zaki et al., 1983& de Varies et al., 1996). TNF- α also stimulates nitric oxide synthase located over cerebral endothelial tissues to generate nitric oxide40 Moreover, high levels of TNF-α can produce a variety of metabolic derangements including accumulation of gamma-aminobutyric acid (Minuk, 1986), endogenous benzodiazepine (Bourdiol et al., 1991), and false neurotransmitters (Freund, 1987). The above mediators may further aggravate the neurologicaldys function observed in hepatic encephalopathy (Chu et al., 2001).

The Plfext effects on oxidative stress in the brain in TAA-induced HE may be related to the effects of Plfext on AchE activity. Although evident, the role of AchE in the pathogenesis of HE is still not completely understood. Studies in patients with liver cirrhosis have found an increase in AchE activity in the brain (García-Ayllón, et al., 2008), while in TAA-induced model of cirrhosis the activity of AchE was found to be elevated in enthorinal cortex, nc. accumbens, anterodorsal and anteroventral thalamus, and decreased in CA1, CA3 region and dentate gyrus of hippocampus (Méndez et al., 2011). On the other hand, (Zarros et al., 2008) have not found changes in AchE activity in acute HE, while (Swapna et al., 2007), in contrast to our study, have observed a decline in this enzyme activity in the cortex after acute TAA administration. These discrepancies may be explained by different doses of TAA used in these studies and partly by different mechanisms of HE development in various models. The complexity of AchE effects on the pathogenesis of HE may be further confirmed by improvement of cognitive, but not motor functions, after AchE inhibition by rivastigmin in rat liver failure (García-Ayllón et al., 2008).

hepatocytes, the reduction in SH-protein bond production, the disturbance in the endocrine. The present study evaluated the effects of Fructus Piperis Longi ethanol extract (Plfext) treatment on hepatic encephalopathy (HE) resulting from TAA administration. TAA treatment caused a significant increase in the activity levels of serum ALT and AST, and Plfext reduced those levels. Histopathological data also

point toward a protective effect of Plfext against TAA-induced HE.

Histopathological analysis showed that lesions of periportal hepatic cells with periportal necrosis and macrophage infiltration in the TAA group were ameliorated in rats receiving Plfext following the induction of liver damage. Nitric oxide (NO) is a potent biological mediator produced by hepatocytes after exposure to cytokines (Geller et al., 1994, Kangetet al., 2002) that influences physiological processes in every organ and tissue (Yao, et al., 2009). It is rapidly oxidized in blood and tissues to form nitrate and nitrite (Shaker, et al., 2010). Production of NO can be determined by measuring liver nitrite and nitrate levels (Yao, et al., 2009), and its activity is regulated at the transcriptional level by cytokines and the exposure of cells to other inflammatory stimuli such as endotoxin or ROS (Manjeet, et al., 1999). The indiscriminate destruction of cells and tissues by NO and its reactive nitrogen intermediates may be involved in the pathology of many inflammatory disorders, such as sepsis and severe gastroenteritis, where the levels of nitrate and nitrite are greatly increased (Moncada et al., 1993, Herulf, et al., 1999, Crawford, et al., 2004). Fructus Piperis Longi has recently been proposed as a chemo-preventive agent for its antioxidant activities (Amonkar et al., 1986, Padma et al., 1989).

The present study showed that treatment of Plfext significantly reduced HE as evidenced by enhancement of the antioxidant status and improvement of liver functions. The results are consistent with other reports on the role of polyphenols against oxidative stress (Chen et al., 2002) because Plfext is rich in polyphenols (Jeng et al., 1994) which may up-regulate the antioxidant (Lei et al., 2003, Jeng et al., 2004), thereby decreasing the free radical-induced lipid peroxidation (Choudhary et al., 2002).

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