Research Article

Propylthiouracil-Induced Liver Injury in Mice and the Protective Role of Taurine

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A B S T R A C T

Background: Propylthiouracil (PTU) is a thionamide drug used in the management of hyperthyroidism in human. On the other hands, several cases of hepatotoxicity, hepatic failure and even death have been reported after PTU administration. No specific protective agent has been developed against this complication yet. Taurine is a sulfur containing amino acid which its beneficial effects in liver tissue has been reported in previous studies. This study was designed to investigate the effect of taurine on PTU-induced liver injury. **Methods:** Mice received PTU (100 mg/kg, oral) and different doses of taurine (250, 500 and 1000 mg/kg, i.p, administered 2 hours after PTU) and markers of liver injury were monitored. **Results:** Acute exposure to PTU caused hepatotoxicity in mice as evidenced by increase in plasmatic alanine aminotransferase (ALT), occurrence of significant lipid peroxidation, and hepatic glutathione depletion. The mentioned changes were endorsed by histopathological lesions of liver which were mainly manifested as pre-portal inflammation. Taurine administration (500 and 1000 mg/kg, i.p) resulted in reduction of lipid peroxidation, showed rebalancing effect on liver GSH level, and normalized plasma ALT. Taurine administration didn't affect PTU-induced inflammatory cell aggregation in liver. **Conclusion:** In view of these results, taurine seems to exert some beneficial effects against PTU-induced liver injury.

Introduction

Propylthiouracil (PTU) is a thionamide derivative used in the management of hyperthyroidism.[1] However, PTU-induced hepatotoxicity becomes a clinical challenge, since several cases of liver injury and even hepatic failure and/or death have been reported after its administration.[2-5] PTU-induced hepatotoxicity is especially a deleterious adverse effect in pediatrics.[6] Some recent investigations suggested withdrawing PTU from the market [5], and/or using this drug only in special cases where its administration is inevitable.[7] The mechanism(s) by which PTU causes hepatic injury is not clear yet.[8-10] Previous studies suggest that the nature of PTU-induced hepatotoxicity is hepatocellular rather than cholestatic.[11] Some investigations observed the involvement of mitochondrial injury in PTU-induced hepatic damage.[12] The role of metabolism and pharmacokinetic parameters are also indicated to be involved in PTU-induced liver-injury.[8,13] Moreover, defect in cellular defence mechanisms might be involved in PTU toxicity. On the other hands, there is no specific therapeutic agent against PTU-induced liver injury.

Taurine (2-aminethaneosulfonic acid), is a non-essential amino acid found in daily dietary intake of humans.[14] A wide range of different physiological roles are attributed to taurine [15], including neuroprotection[16], antiepileptic effect[17], cardio-protection[18], and protection against diabetes-related complications.[19,20] Furthermore, it has been founded that this amino acid protected liver from different xenobiotics-induced hepatotoxicity, including several drugs. [9,21-27]
Taurine might provide hepatoprotection by a broad spectrum of mechanisms. It has been found that this amino acid had antioxidant and radical scavenging properties.[28] Moreover, taurine plays as a cellular osmoregulator [29] and keeps cellular calcium ion (Ca^{2+}) hemostasis.[30] Antioxidant properties and the ability of this amino acid on the induction of different cellular defense mechanisms, including enzymes such as glutathione peroxidase (GPx), glutathione transferase (GST), catalase (CAT), and superoxide dismutase (SOD) have been proved.[31,32] Some studies have shown that taurine effectively protected crucial intracellular organelles such as mitochondria against stresses.[30,33,34] Several other investigations revealed that taurine was

able to modulate immune system-mediated toxicities.[35] All these unique properties of taurine, in addition to its safety even in very high doses[36-38], make this amino acid a potential therapeutic option against xenobiotics-induced toxicity in different organs, including liver.

The aim of current study was to evaluate the effect of taurine against PTU-induced liver injury. Different doses of PTU alone and/or in combination with taurine were administered and the serum level of ALT as a specific marker of liver injury, liver glutathione reservoirs, and tissue lipid peroxidation were monitored. After all, liver histopathological changes were assessed to evaluate the hepatoprotective effect of taurine against PTU-induced injury. In current investigation, NAC was used as a positive control since its protective effects on isoniazid-induced hepatotoxicity has been proven in previous investigations.[39,40]

Material and Methods
Chemicals
5,5′-dithionitrobenzoic acid (DTNB), reduced glutathione (GSH), Taurine, Malondialdehyde, and n-butanol were purchased from Sigma-Aldrich (St. Louis, USA). Thiobarbituric acid (TBA), 6-propyl-2-thiouracil (Propylthiouracil, PTU), Trichloro acetic acid (TCA), meta-Phosphoric acid, Methanol, N-acetyl cysteine, and hydroxy methyl amino methane (Tris) were purchased from Merck (Dardamstd, Germany). The kit for liver biochemistry analysis (ALT), was obtained from Pars Azmun ® Company (Tehran, Iran). All salts for preparing buffer solutions were of the highest grade commercially available.

Animals
Male Swiss albino mice, 6 weeks old (25-35 g weight), were obtained from Animal Breeding center of Shiraz University of Medical Sciences (Shiraz, Iran). Mice were housed in cages on wood bedding at a temperature of 25 ± 3 °C. Animals had free access to food and water. The animals received humane care and use, and were handled according to the animal handling protocol at Shiraz University of Medical Sciences, approved by a local ethics committee.

Mice were randomly divided equally into seven groups of six animals. PTU was given by gavage. Taurine and NAC were administered intraperitoneally. All agents were dissolved in 0.9% saline. The treatments were as follow:
A) Control; vehicle-treated (0.9% saline solution) only.
B) 25 mg/kg of PTU.
C) 50 mg/kg of PTU.
D) 100 mg/kg of PTU.
E) 100 mg/kg of PTU + Taurine (250 mg/kg).
F) 100 mg/kg of PTU + Taurine (500 mg/kg,).
G) 100 mg/kg of PTU + Taurine (1g/kg).
H) 100 mg/kg of PTU + NAC (300 mg/kg).
I) 100 mg/kg of PTU + NAC (500 mg/kg).

Taurine was administered two hours after PTU in all experiments. No significant toxicity with taurine was observed in current study when administered alone at mentioned doses.

Plasma biochemical analysis and liver histopathology
Blood was collected from the abdominal *vena cava* under pentobarbital anesthesia (60 mg/kg, i.p), and the liver was removed. The blood was transferred to anticoagulant coated tubes and plasma was prepared by centrifugation. Plasma alanine transaminase (ALT) activity was measured using commercial kits. For histopathological evaluation, samples of liver were fixed in buffered formalin solution (0.4% sodium phosphate monobasic, NaH_2PO_4, 0.64% sodium phosphate dibasic, Na_2HPO_4, and 10% formaldehyde in distilled water). Paraffin-embedded sections of liver were prepared and stained with haematoxylin and eosin (H&E) before light microscope viewing.[41]

Liver glutathione content
The glutathione contents of mice liver were assessed by determining non-protein sulphydryl contents with the Ellman reagent.[42] Liver samples (200 mg) were homogenized in 8 ml of cooled EDTA solution (0.02 M). Then, 5 mL of liver homogenate was mixed with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA). The mixture was shaked and then centrifuged (765 *g*, 15 minutes, at 4°C). Then, 2 mL of supernatant was added to 4 mL of cooled Tris buffer (pH= 8.9) and 100 μL of DTNB solution (0.01 M in methanol).[43] The absorbance of developed color was read at 412 nm using an Ultrospec 2000®UV spectrophotometer.

Lipid peroxidation
Level of lipid peroxidation in mice liver was assessed by thiobarbituric acid reactive substances (TBARS) test.[44] Briefly, reaction mixture consists of 0.5 mL of 10% liver homogenate, 3 ml phosphoric acid 1% and 1 mL of 1% thiobarbituric acid (TBA). The mixture was shaked, then heated in boiling water (100°C) for 45 minutes. Afterwards, 4 mL of n-butanol was added to reaction mixture and vigorously shaked. After centrifugation in 765*g* for 5 minutes, the absorbance of developed color in n-butanol phase was read at 532 nm using an Ultrospec 2000®UV spectrophotometer.[45]

Statistical Analysis
Results are shown as Mean±SEM for six animals. Comparisons between multiple groups were made by a one-way analysis of variance (ANOVA) followed by Turkey's *post hoc* test. Differences were considered significant when $p<0.05$.

Results
PTU was administered to mice in different doses (50, 100 and 200 mg/kg, oral) (Figure 1), and plasma ALT level was monitored at different time intervals (Figure 1). It was found that 100 mg/kg of PTU caused a peak

in plasmatic ALT content (>3 ULN, which indicates hepatic injury [46]), six hours after drug administration (Figure 1). This elevation in ALT was significantly higher than control (vehicle-treated) animals (Figure 1).

The appropriate toxic dose and time for future experiments was obtained through this estimation (100 mg/kg after 6 hours) (Figure 1).
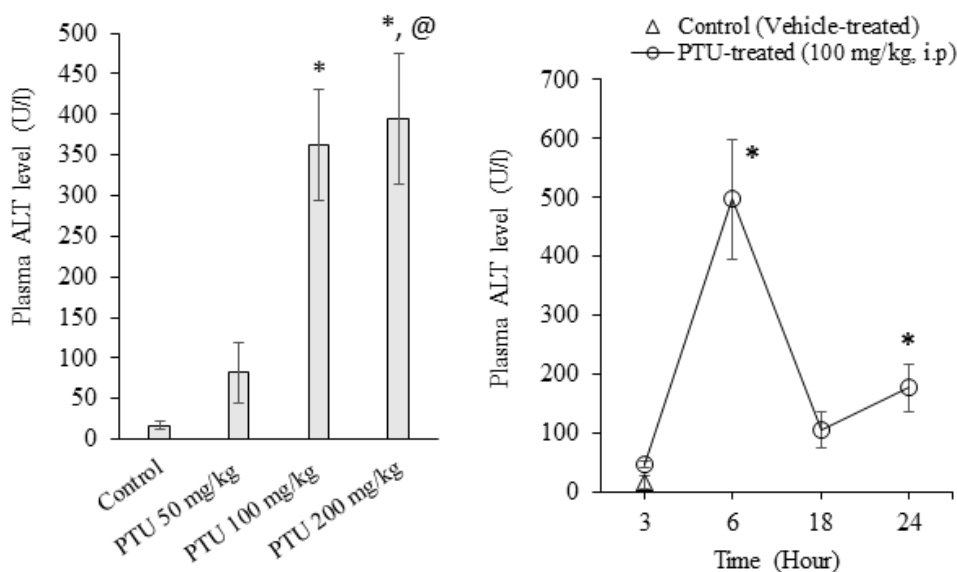

Figure 1. Dose and time-response of PTU-induced elevation in plasma ALT levels in mice.
Data are shown as Mean±SEM four six animals. PTU: propylthiouracil.
*Indicates significantly higher as compared to control animals ($p < 0.05$).
@ Two out of six animals were dead after PTU administration.

PTU (100 mg/kg, oral) caused significant liver injury in mice as judged by significant elevation in plasma ALT (Figure 2), lipid peroxidation (Figure 3), depletion of liver tissue glutathione (Figure 4), and finally histopathological lesions which was presented as inflammatory cells aggregation in mice liver (Figure 5).
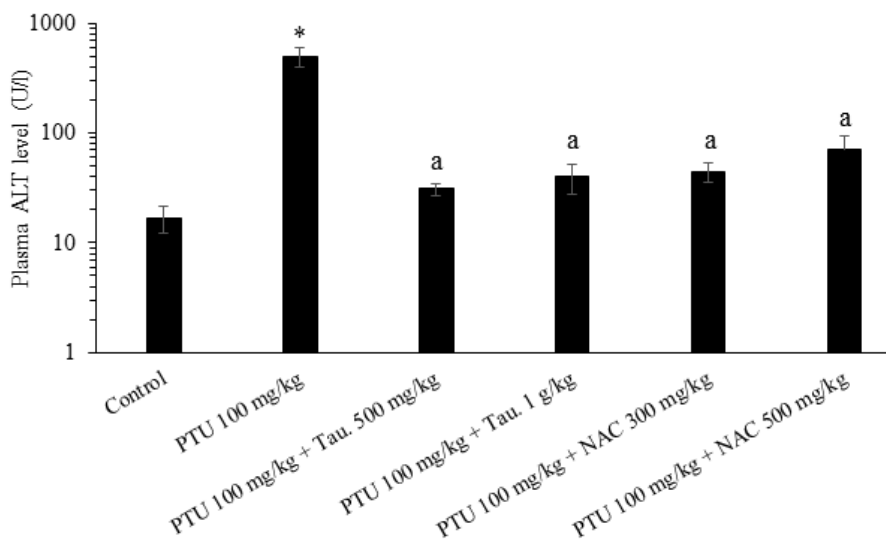
Figure 2. Plasma ALT level in PTU-challenged group and the role of taurine and/or NAC administration. PTU: propylthiouracil. NAC: N-acetylcysteine.
Data are given as Mean±SEM for 6 animals in each group.
*Indicates significantly higher as compared with control animals ($p < 0.05$).
a Shows significant decrease as compared to PTU-treated animals ($p < 0.05$).
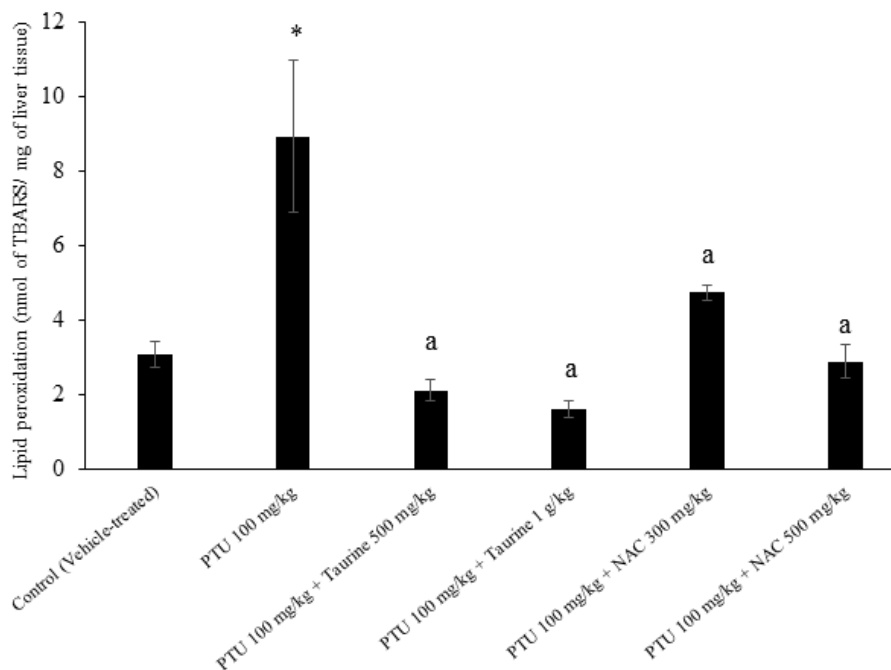
Figure 3. PTU-induced lipid peroxidation in mice liver. PTU: propylthiouracil. NAC: N-acetylcysteine.
Data are shown as Mean±SEM for six animals in each group.
*Indicates significantly higher as compared to control group ($p<0.05$).
[a] Indicates significant changes as compared to PTU-treated animals ($p<0.05$).
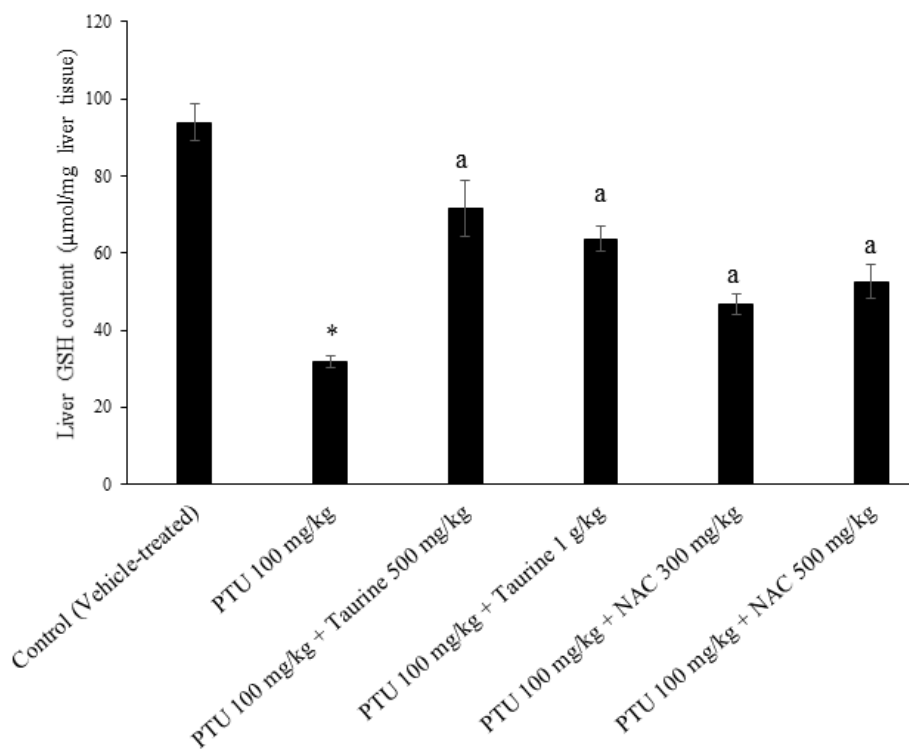
Figure 4. Effect of PTU on hepatic glutathione reservoirs.
PTU: propylthiouracil. NAC: N-acetylcysteine.
Data are presented as Mean±SEM for six animals in each group.
*Indicates significant decrease as compared to control group ($p<0.05$).
[a] Indicates significant change as compared to PTU-treated animals ($p<0.05$).
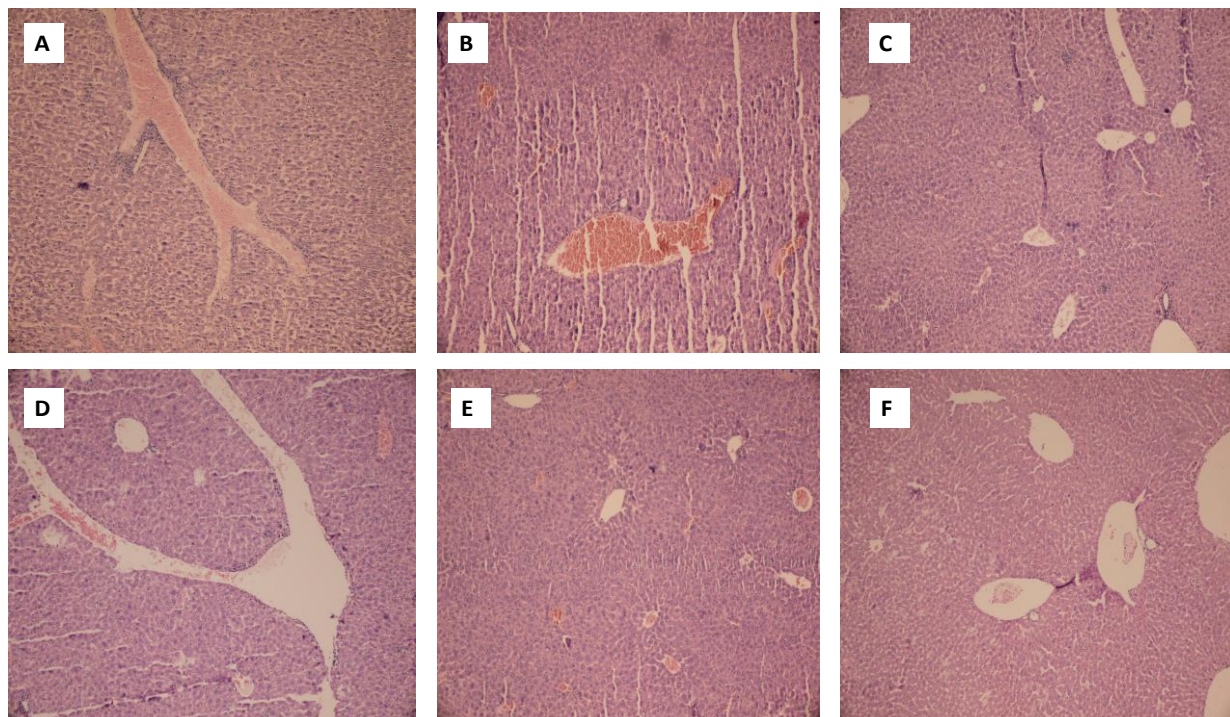
Figure 5. Histopathological evaluation of mice liver in PTU-treated animals and the role of taurine administration.
A: Mice normal liver histopathology. **B**: PTU-treated (100 mg/kg, gavage) animals. Liver tissue necrosis, sinusoidal dilation, and inflammatory cells aggregation is presented in this group. **C & D**: PTU (100 mg/kg, gavage) + Taurine (500 mg/kg and 1000 mg/kg, i.p. respectively). **E & F**: PTU (100 mg/kg, gavage) + NAC (300 mg/kg and 500 mg/kg, i.p. respectively). Taurine didn't affect inflammatory cells aggregation in PTU-treated animals (**C & D**).

Different doses of taurine (500, and 1000 mg/kg, i.p) were administered to find a proper hepatoprotective dose of this amino acid against PTU-induced liver injury. It was found that, administration of 500 and 1000 mg/kg of taurine effectively alleviated all the toxicity markers of PTU-induced liver injury in mice. Administration of 250 mg/kg of taurine couldn't significantly protect liver against PTU-induced injury. The hepatoprotective properties of taurine (500 and 1000 mg/kg, i.p.) against PTU-induced hepatic injury was revealed by suppressing lipid peroxidation (Figure 2), preventing liver glutathione depletion (Figure 3), and a significant decrease in plasmatic ALT levels. However, taurine (500 and 1000 mg/kg) could not affect PTU-induced pre-portal inflammation in liver. NAC administration (300 and 500 mg/kg, i.p.), also effectively alleviated all hepatic adverse events associated with PTU, in this investigation(Figures 1-4).

Discussion

PTU administration caused hepatic injury as evidenced by high plasma level of ALT, significant hepatic glutathione depletion, lipid peroxidation and tissue histopathological changes. Taurine (500 and 1000 mg/kg, i.p) treatment effectively diminished PTU-induced liver injury in mice.
GSH serves as a critical intracellular defense mechanism against xenobiotics-induced toxicity.[47] Therefore reduction in GSH reservoirs can impair the cell defense capacity which might lead to cellular injury and death. In the present study, a decrease in liver GSH content was observed in PTU-exposed animals. Examination of the effect of taurine treatment revealed that an increased in hepatic GSH content was observed when this amino acid was administered after PTU. Depletion of GSH following PTU exposure may be due to induction of oxidative stress and also consumption of GSH. It has been found that PTU and/or its metabolites inhibited glutathione transferase (GST) or glutathione peroxidase (GPx) enzymes dose dependently.[48] These enzymes are responsible for keeping glutathione in its reduced state and conjugating xenobiotics by GSH. Hence, the reduction in cellular GSH level in PTU-treated animals might be attributed to the effects of this drug on the mentioned enzymatic systems.
We found that PTU caused lipid peroxidation in liver tissue. Lipid peroxidation is a common future of ROS formation and oxidative stress in liver. Taurine administration effectively mitigated PTU-induced lipid peroxidation. Therefore the beneficial role of taurine in the present study may be, at least in part, due to the effect of this amino acid on lipid peroxidation and glutathione depletion induced by PTU.
Although taurine didn't affect PTU-induced pre-portal inflammation and inflammatory cells aggregation in mice liver, it poses hepatoprotection against this drug, since taurine administration significantly alleviated plasma level of ALT, decreased tissue lipid peroxidation and prevented hepatic glutathione

depletion. It has been reported that taurine could effectively suppress immune cells including neutrophils and macrophages.[35] The immune cell suppressive properties of taurine might be involved in its hepatoprotective properties in current investigation.

Numerous studies have reported the beneficial effects of taurine against xenobiotics-induced liver injury, including different drugs. Taurine not only can directly scavenge free radicals but also stimulates the activity of enzymes involved in the cellular defense mechanisms. Taurine is an important organic osmolyte.[29] This amino acid is able to prevent cell swelling and/or shrinkage by regulating ion channels and transporters.[29] The osmoregulatory effect of taurine might also be involved in its protective properties against PTU.

Taurine has been found to be an enzyme inhibitor.[49] Although the precise metabolic pathways of PTU has not been cleared, the enzyme inhibitory effect of taurine might also be potentially involved in its hepatoprotective mechanisms.

Overall, we might be able to conclude that, taurine protected mice liver through its ability in modulating lipid peroxidation and preventing decline in hepatic glutathione reservoirs. An understanding of the precise protective mechanism of taurine against PTU-induced hepatotoxicity and its beneficial effects against other drugs-induced liver injury remain to be further determined.

Acknowledgements

Conflict of Interest

The authors report no conflicts of interest.

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