Research Article

Tiron Mitigates Thioacetamide-Induced Acute Liver Injury


Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

ARTICLE INFO

Acute liver injury is a crippling disorder accompanied by extensive impairment of liver’s synthetic, metabolic and detoxifying functions. Tiron is a synthetic vitamin E analog, proven to be anti-oxidant. This study was undertaken to investigate the protective activity of tiron against thioacetamide (TAA)-induced acute liver injury. Rats were orally treated with tiron (300 mg/kg) for eight days prior to I.V. TAA either (70 mg/kg) or (280 mg/kg) to induce acute liver injury. Biochemical evaluation of hepatotoxicity indices, oxidative stress, cytotoxicity and inflammatory marker: interleukin-6 (IL-6) was conducted along with histopathological examination. Meanwhile, tiron was found to mitigate the TAA-induced elevation of ALT, AST and ALP. However, serum albumin levels mildly improved. Tiron restored liver GSH contents and maintained liver SOD activity. Moreover, tiron significantly reduced the level of serum IL-6. In context, histopathological examination revealed that tiron slightly reduced the extent of TAA-induced necrosis. Tiron has manifested the observed hepatoprotective effect probably by manipulating inflammatory response of liver to injury via downregulating the expression of inflammatory IL-6 and alleviating oxidative stress.

Key words: tiron, thioacetamide, acute liver injury, oxidative stress, necrosis, Interleukin-6.

1. Introduction

The liver is a vital organ that performs a wide array of functions including biotransformation and metabolism and excretion of endogenous and exogenous compounds, synthesis of various plasma proteins and storage of glycogen (Cemek et al., 2010).

Acute liver failure (ALF) is a debilitating disorder that can quickly result in multiorgan failure and death (Bernal and Wendon, 1999). Viral-induced hepatitis and drug or toxin-induced liver injuries are among the main causes of ALF (Gill and Sterling, 2001). ALF is characterized by loss of hepatic mass and deterioration of hepatocytes functions which may result in coma and death if not managed right away (Newsome et al., 2000).

The human body cannot dispense with inflammation as an immune system response to any injury. Although inflammation combats virulent microbes and toxins, it can inflict detrimental impacts on the host (Medzhitov, 2010). Therefore, regulation of cytokine expression is a major concern when tackling inflammation (Dayer, 2003). Typically, increased oxidative stress has been claimed to induce cytotoxicity (Han et al., 2006).

Thioacetamide is a hepatotoxin which has been repeatedly utilized to experimentally induce ALF in rats (Bautista et al., 2010; Chen et al., 2008; Shapiro et al., 2006). TAA has been reported to induce acute liver injury, liver fibrosis, cirrhosis and hepatocellular carcinoma and to mimic the features of hepatitis C infection in human (Abdelaziz et al., 2015; Alshawsh et al., 2011; Mormone et al., 2012; Said et al., 2013). Two dose levels of TAA 70 mg/kg and 280 mg/kg were used to induce liver injury to assess the hepatoprotective potential of tiron against different extents of TAA-induced acute liver injuries.

Tiron is a synthetic analog of vitamin E. However, tiron is water-soluble in contrast to vitamin E (Zafarullah et al., 2003). Vitamin E has reportedly demonstrated protection against experimentally induced non-alcoholic steatohepatitis via improving liver functions and mitigating the inflammatory response in liver (Gitto et al., 2015).
Tiron has been reported to be an effective antidote for vanadyl sulphate intoxication in liver and kidney of mice (Gomez et al., 1991), and to mitigate bortezomib-induced poly (ADP-ribose) polymerase protein breakage and apoptosis in human lung cancer cells (Ling et al., 2003). Also, tiron has shown anti-apoptotic effect on melanoma cells in humans (Yang et al., 2007).

The current investigation was undertaken to assess the hepatoprotective potential of tiron against thioacetamide-induced acute liver failure. Hepatocytes’ integrity, hepatic synthetic and excretory functions were evaluated. Serum LDH and serum IL-6 were measured to assess cytoprotective and anti-inflammatory potentials of tiron. Hepatic reduced glutathione contents (GSH) and superoxide dismutase (SOD) activity were determined. Lastly, histopathological examination of liver specimens was carried out.

2. Materials and methods

2.1. Animals

Thirty adult male Sprague Dawely rats; 8 weeks age weighing about 200 ± 20 g, were bought from the Experimental Research Centre of Nephrology and Urology Center, Mansoura University, Egypt. The experimental protocol complies with the ethics and guidelines of care and handling of experimental animals adopted by "The research Ethics Committee", Faculty of Pharmacy, Mansoura University.

2.2. Chemicals and reagents

Tiron was purchased (Sigma-Aldrich, St. Louis, MO, USA), and was suspended in 0.5% carboxymethylcellulose (CMC) for oral administration. Thioacetamide was purchased (Sigma-Aldrich, St. Louis, MO, USA) and dissolved in 0.9% w/v NaCl (saline) for I.V. injection.

2.3. Experimental Design

Acute liver injury was induced in rats, by single I.V injection of TAA (70 mg/kg) and TAA (280 mg/kg), respectively (Chen et al., 2008). Rats were randomly distributed into 5 groups each comprised 6 rats. Group (1): Negative control: rats received the vehicle (0.2 ml of 0.5% CMC, orally) once daily for 8 days. Group (2): TAA1 group: rats received 0.2 ml of 0.5% CMC, orally and on 8th day of the experiment TAA (70 mg/kg, I.V.) (Chen et al., 2008). Group (3): TAA2 group: rats received 0.2 ml of 0.5% CMC, orally and on 8th day of the experiment TAA (280 mg/kg, I.V.) (Chen et al., 2008). Group (4): Tiron1 group: rats received tiron (300 mg/kg, orally) (Bowes and Thiemermann, 1998) + TAA (70 mg/kg, I.V.) on the 8th day of the experiment (Chen et al., 2008). Group (5): Tiron2 group: rats received tiron (300 mg/kg, orally) (Bowes and Thiemermann, 1998) + TAA (280 mg/kg, I.V.) on the 8th day of the experiment (Chen et al., 2008).

Twenty four hours after TAA injection; all the rats were sacrificed via injection of an overdose of thiopental sodium (40 mg/kg). Blood samples were compiled from the rats under thiopental anesthesia via puncture of the retro-orbital plexus. The serum was separated and used promptly to estimate the biochemical parameters.

Livers from all rats were collected, rinsed and weighed to calculate liver/body weight index. The left lobe of the liver was immediately preserved in formalin for histopathological examination and the right lobe of the liver was used for preparation of liver homogenate.

2.4. Preparation of liver homogenate

500 mg of the right lobe of the liver was weighed, homogenized in 1.15% KCl and centrifuged to yield 10% of liver homogenate. The supernatant was separated and referred to as liver homogenate and it was used to estimate ant-oxidant biomarkers: GSH and SOD.

2.5. Assessment of serum ALT, AST, albumin, ALP and LDH

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin were determined according to manufacturer’s instruction using commercially available kits (Biomerieux, Marcey-L’Etoile, France). Serum ALP levels were measured using commercially available kits (ABC Diagnostics, Egypt). Serum LDH level was determined using the commercially available kit (biomed. Diagnostics, Egypt). Test procedures were conducted according to the provided manufacturer’s instructions.

2.6. Assessment of hepatic content of GSH and hepatic SOD activity

SOD activity and GSH content in liver homogenate were determined using commercially available kit (Biodiagnostic, Giza, Egypt). Test procedures were undertaken in accordance with the provided manufacturer’s instructions.

2.7. Assessment of serum IL-6 levels

Serum IL-6 was measured using commercial ELISA assay kit (RayBio, Norcross, USA). The concentration was expressed as pg/ml. methodology of determination was conducted according to the supplied manufacturer’s instructions.

2.8. Histopathological examination

The left lobe of the livers from all rats were collected and dipped in ice-cold saline solution, fixed in 10% neutral buffered formalin solution. Specimens were immersed in paraffin and cut into sections (4 to 5 μm) thickness slides. The slides were stained with hematoxylin and eosin (H&E) and examined under a microscope randomly and the histopathologist was blinded to the experimental groups.

2.9. Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey Kramer’s test was run, where data was demonstrated as mean ± standard error (SE). GraphPad Instat version 3.06 software program was used to statistically analyze data. Graphs were drawn by GraphPad Prism (ISI® software, USA) version 5 software.
3. Results and Discussion

Effect of tiron (300 mg/kg) administration orally for 8 days on TAA (70 mg/kg) and TAA (280 mg/kg) induced acute liver injury.

3.1. Liver/body weight Index:
I.V. TAA (70 mg/kg) and TAA (280 mg/kg) significantly increased liver/body weight indices by approximately 1.2 and 1.15 fold respectively in comparison to healthy control group, figure (1). Oral tiron (300 mg/kg) for 8 days substantially reduced the liver/body weight index by nearly 10% in comparison to TAA (70 mg/kg) diseased control. Conversely, oral tiron (300 mg/kg) for 8 days failed to produce a significant reduction in the liver/body weight index when compared to TAA (280 mg/kg) diseased control, figure (1).

3.2. Serum ALT and AST:
Significant elevation in serum ALT and AST, by about 21% and 44% in comparison to TAA (280 mg/kg) diseased control, table (1).

3.3. Serum albumin and ALP
As shown in table (1), I.V. TAA (70 mg/kg) and (280 mg/kg) markedly reduced serum albumin levels to about three quarters (3/4) and two thirds (2/3) the value of albumin in the healthy control groups respectively.

Oral tiron (300 mg/kg) once daily for 8 days failed to elicit a remarkable increase in serum albumin compared to either TAA (70 mg/kg) or TAA (280 mg/kg) diseased groups, table (1).

In context, as illustrated in table (1), I.V. TAA (70 mg/kg) and (280 mg/kg) elicited approximately a 1.3 and 1.6 fold significant increase in serum ALP respectively in comparison to the healthy control group. Daily oral tiron (300 mg/kg) for 8 days significantly lowered the increased serum ALP levels by 17.8% and 38% in comparison to TAA (70 mg/kg) and TAA (280 mg/kg) diseased groups respectively, table (1).

3.4. Serum LDH
As represented in table (2), I.V. TAA (70 mg/kg) and (280 mg/kg) resulted in a 1.8 and 1.6 fold increase respectively in serum LDH level in comparison to negative control group.

Oral tiron (300 mg/kg) for 8 days prior to TAA (70 mg/kg) restored serum LDH to normal levels, table (2).
Figure (1): a. Effect of tiron (300 mg/kg) administration orally for 8 days on liver/body weight index; b. Effect of tiron (300 mg/kg) administration orally for 8 days on hepatic GSH content; c. Effect of tiron (300 mg/kg) administration orally for 8 days on hepatic SOD activity.

Tiron (300 mg/kg) was orally administered for 8 days prior to I.V. Thioacetamide (70 mg/kg) or (280 mg/kg). Rats were sacrificed 24 hours later. Data are expressed as the mean ± SEM (n=6). Statistical analysis was performed using ANOVA followed by Tukey-Kramer test. *: Significantly different vs. negative control at P<0.05; †: Significantly different vs. corresponding TAA1 group at P<0.05; #: Significantly different vs. corresponding TAA2 group at P<0.05.

However, daily oral tiron (300 mg/kg) decreased serum LDH levels by nearly 18% in comparison to TAA (280 mg/kg), table (2).

3.5. Serum IL-6

I.V. TAA (70 mg/kg) and (280 mg/kg) significantly elevated serum IL-6 levels by approximately 72.5% and 110% respectively in comparison to negative control groups, table (2).

Daily oral administration of tiron (300 mg/kg) for 8 days remarkably reduced serum IL-6 by about 16% and 51% in comparison to TAA (70 mg/kg) and TAA (280 mg/kg) diseased controls respectively, table (2).

3.6. Hepatic SOD activity and GSH contents

Liver GSH content vigorously declined by 59% and 81% in TAA (70 mg/kg) and (280 mg/kg) diseased controls respectively compared to negative control group figure (2).

Oral pre-treatment with tiron (300 mg/kg) slightly increased GSH by nearly 39%, but in a non-significant way compared to TAA (70 mg/kg) diseased controls, figure (2). On the other hand, daily oral tiron (300 mg/kg) for 8 days significantly elevated GSH levels by 120% in comparison to the TAA (280 mg/kg) diseased group.

In context, single I.V. TAA (70 mg/kg) and (280 mg/kg) significantly diminished liver SOD activity by approximately 61% and 82% respectively in comparison to healthy control rats, figure (3). Oral tiron (300 mg/kg) for 8 days substantially protected against TAA-intoxication and restored SOD to nearly basal levels in comparison to TAA diseased controls, figure (3).

3.7. Histopathological examination

Histopathological examination of liver specimens of the negative control group stained with H&E revealed normal hepatocytes’ structure with absence of any evidence of injury or necro-inflammatory changes (figure 4: A).

Conversely, examination of both TAA (70 mg/kg) and TAA (280 mg/kg) diseased controls specimen revealed massive bridging necrosis and centrilobular necrosis with more extensive injury in TAA (280 mg/kg) evident in increased portal inflammation and significant tissue infiltration with inflammatory cells (figure 4: B, C).

Treatment with tiron (300 mg/kg) prior to either TAA (70 mg/kg) or TAA (280 mg/kg) revealed slight reduction in necrosis with persistence of mild microvesicular steatosis, centrilobular necrosis and moderate portal inflammatory infiltrate (figure 5: E, F).

The current study sheds light on the potential significant hepatoprotective efficacy of tiron. Oral tiron administration in the current study resulted in improvement of biochemical parameters under investigation. It significantly improved liver functions and LDH. Serum albumin also improved, but not in a significant manner. Meanwhile, serum ALP significantly improved. Also, serum IL-6 significantly declined with tiron treatment. Hepatic SOD activity and GSH liver contents significantly increased after tiron treatment. Histopathological examination also revealed concomitant tissue improvement retraction of TAA-induced necrosis.

To date, this is the first study which investigates the potential hepatoprotective influence of tiron against TAA-induced acute liver injury and failure. Tiron has been proven to show protective efficacy against acetaminophen-induced acute liver injury and hepatotoxicity (Shoeib et al., 2015).

Acute liver failure (ALF) is a complex multisystemic illness. It usually develops after severe insult to the liver. ALF is characterized by severe impairment of liver synthetic and metabolizing functions (Trey and Davidson, 1970).
Commonly, TAA has been administered intraperitoneally to induce ALF (Bautista et al., 2010). In the current study, TAA was administered intravenously. Chen et al. proposed that I.V. injection is superior to I.P. because the single I.V. injection of TAA doesn't induce hepatic encephalopathy, renal failure, hypoglycemia or hypotension that can increase mortality rate (2008).

Thioacetamide has been claimed to increase hepatocytes’ permeability which explains the release of cell content and the elevation in biochemical parameters: ALT, AST, ALP and LDH (Alshawsh et al., 2011).

TAA has also been reported to induce significant centrilobular hepatotoxicity. The oxidative metabolism of TAA yields reactive oxidative agents; thioacetamide sulphoxide and thioacetamide-S, S-dioxide (Chilakapati et al., 2005; Kucera et al., 2011). Thioacetamide-S, S-dioxide is a very reactive compound and causes oxidative injury (Kwon et al., 2010; Porter and Neal, 1978) and necrosis by targeting tissue macromolecules, lipids, protein, and by induction of DNA peroxidation (Alshawsh et al., 2011; Bruck et al., 2004). Furthermore, Thioacetamide-S, S-dioxide has been reported to induce nuclear factor kappa B (NF-kB), also resulting in centrilobular necrosis (Kucera et al., 2011; Shapiro et al., 2006). Moreover, TAA has been reported to deplete several antioxidants that exist in the body (Bautista et al., 2010; Sun et al., 2000).

Reactive oxygen species induce cell damage by induction of peroxidation of membrane lipids, denaturation of proteins -e.g enzymes and ion channels- and DNA injury. Exposing cultured cells to ROS results in strand breakage of DNA and subsequent activation of the nuclear enzyme, poly (ADP-ribose) synthetase (PARS) (Berger, 1985; Szabo et al., 1996). This explains the correlation between oxidative stress, inflammation and acute liver injury.

In the current study, single I.V. injection of both TAA (70 mg/kg) and TAA (280 mg/kg) elicited a significant increase in serum ALT and AST, implying impairment of hepatic integrity and loss of hepatocytes functions.

Serum albumin has been taken an indicator to evaluate the synthetic ability of hepatocytes. Serum
albumin is not altered in mild liver injury, but considerably diminishes in the face of severe liver necrosis (Rosenthal, 1997). Rats receiving single dose of either TAA (70 mg/kg) or (TAA 280 mg/kg) demonstrated an outstanding, significant fall in estimated serum albumin levels confirming the incidence of ALF. The alterations in liver transaminases levels, serum ALP and serum albumin are consistent with previously reported patterns of TAA-induced acute injury (Abdelaziz et al., 2015; Alkiyumi et al., 2012; Chen et al., 2008).

Meanwhile, the massive significant elevation in serum ALP is indicative of disruption of liver’s excretory functions. The level of ALP usually increases in intrahepatic cholestasis and liver’s infiltrative diseases (Gaze, 2007).

Significant elevation in serum LDH is indicative for tissue breakdown, cell membrane damage, loss of cellular contents and cytotoxicity (Balduzzi et al., 2004).

GSH is an important endogenous antioxidant needed to preserve the cells’ regular redox status (Mantawy et al., 2012; Masella et al., 2005). There was substantial decrease in SOD activity and GSH liver content after TAA injection. The decline in SOD activity is in line with Alkiyumi et al. (2012).

The enormous decline in hepatic GSH content is in agreement with previous studies (Akbay et al., 1999; Kucera et al., 2011). TAA-induced hepatocellular injury of rats is partly triggered by increased oxidative stress due to GSH depletion (Akbay et al., 1999; Kucera et al., 2011). Accordingly, it can be proposed that depletion of the intracellular anti-oxidant defense system predisposed hepatocytes to disruption and in turn leakage of LDH.

The inflammatory responses are regulated by proinflammatory mediators such as IL-1 and IL-6 and TNF-α (Medzhitov, 2010). IL-6 release has been linked to cell injury, because IL-6 is considered a primary biomarker in acute inflammatory response (Jean-Baptiste, 2007; Saeed et al., 2012), overproduction of IL-6 has been alleged to result from the acute liver injury and inflammation (Kim et al., 2014; Lacour et al., 2005).

Kupffer cells are considered the primary cells that release pro-inflammatory cytokines such as IL-6 as a response to liver injury or inflammation (Kmiec, 2001; Masubuchi et al., 2003). Serum IL-6 significantly declined following tiron treatment and that may be associated with kupffer cell modulation by tiron.

Interleukin-6 is an activator and product of NF-κB (Hagemann et al., 2008; Robinson and Mann, 2010). IL-6 production is correlated with cell injury and IL-6 is deemed as an initial marker for acute inflammation (Jean-Baptiste, 2007).

NF-κB modulates the inflammatory and immune response in the host by enhancing and expressing particular cellular genes (Cao et al., 2006; Elsharkawy and Mann, 2007). Stimulation of NF-κB is arranged by various signal transduction cascades to overcome diverse stress conditions such as infection and inflammation (O’Dea and Hoffmann, 2009).

In this context, I.V. TAA (70 mg/kg) and TAA (280 mg/kg) dose-dependently elevated IL-6. TAA induced massive bridging necrosis with inflammatory infiltrates mostly of leucocytes. The previous findings were in parallel with Kucera et al. where they found that I.P. TAA (100 mg/kg) increased serum levels of ALT, AST, ALP and IL-6 and caused necrosis in liver tissues upon histopathological examination (Kucera et al., 2011).

Generally, impacts of antioxidants have been widely tested for the prevention and treatment of acute and chronic liver injuries. Some studies revealed that free radical scavengers are extensively beneficial in preventing and treating chronic liver injury (Parola et al., 1992; Shoeib et al., 2015).

Tiron at the investigated dose (300 mg/kg/24h) significantly improved hepatic impairment; particularly it markedly reduced serum ALT. This finding is in line with Bowes and Thiemermann’s study where tiron attenuated the extent of ischemia/reperfusion-induced liver injury (Bowes and Thiemermann, 1998). Interestingly, tiron has been reported to confer significant protection against acetaminophen-induced acute liver injury in rats (Shoeib et al., 2015).

GSH liver contents and SOD activity in liver homogenate remarkably increased, with reflection on liver histopathology. The improvements in ALT, ALP, albumin, hepatic GSH content and histopathological examination are similar to the study of Shrivastava and his colleagues (2007), where tiron demonstrated a profound protective efficacy against vanadium-induced hepatic intoxication in rats (Shrivastava et al., 2007).

Tiron has been reported to inhibit oxaliplatin-induced cell death in colorectal cancer cells (Shi et al., 2012). Furthermore, tiron alleviated oxidative stress markers: O₂⁺ and MDA following ischemia/reperfusion-induced acute liver injury (Bowes and Thiemermann, 1998).

Tiron has also been reported to promote survival of melanoma cell via mitochondria-dependent and ROS-independent mechanisms, involving NF-κB. Interestingly, it has been reported that tiron chemically enhances NF-κB -dependent gene transcription with antiapoptotic effects (Yang et al., 2007). Similarly, some studies revealed that there was a strong correlation between tiron and the activation of NF-κB in melanoma models (Schwartz and Davidson, 2004). ROS has been reported to enhance NF-κB by either the activating protein kinase C pathway or modulating IL-6 expression (Ogata et al., 2000).

Tiron has been also reported to substantially reverse the increase of TnI or phospho-GATA-4 protein expression in neonatal rat cardiomyocytes and H9c2 cells caused by a high glucose concentration which helped to improve myocardial contractility mainly by scavenging ROS (Ku et al., 2011).

Tiron may have demonstrated such cytoprotective and hepatoprotective efficacy by either reducing the generation of free radicals as previously reported (Koyama et al., 2013; Shrivastava et al., 2007) or by reducing inflammatory cells infiltration (Yang et al., 2007).
5. Conclusion:
Acute liver injury is a debilitating disorder that is associated with loss of synthetic and detoxifying functions of liver and can quickly lead to multiple organ failure and death. Tiron is a potential cytoprotective drug that acts mainly through inhibiting inflammatory response mediated by kupffer cells and alleviating oxidative stress. In the current study, tiron improved liver function and decreased cytotoxicity and inflammation. Tiron mildly improved liver's synthetic function and substantially improved hepatic excretory functions. Tiron dramatically improved anti-oxidative defense system. The findings suggest that the use of tiron can be potentially beneficial in prevention of acute liver injury and that it has anti-inflammatory and anti-oxidant effects.

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7. Reference list


