ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Protective effects of vitamin E on experimentally induced hepatotoxicity in rats

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Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 22 July 2015
Published Online: August 2015

Key words:

D-GalN, LPS, ROS, GSH, vitamin E

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Abstract

D-galactosamine (D-GalN) and lipopolysaccharide (LPS) administration lead to increase production of reactive oxygen species (ROS). Increased oxidative stress caused injury of liver cells and therefore increased leakage of liver cell enzymes as alanine transaminase (ALT) and aspartate transaminase (AST) into the systemic circulation. Increased ROS productions also, lead to decreased non-enzymatic antioxidant such as glutathione (GSH). Vitamin E used in this study as it is a powerful antioxidants.This study was designed to elucidate the protective effectofvitamin E on D-GalN/LPS induced hepatotoxicity in albino Wister rats. Acute hepatic failureinduced by intraperitoneal injection of 500 mg/kg D-GalN and 50 µg/kg LPS body weight of adult albino rats.Vitamin E was used as prophylaxis in doses of 400 mg/Kg body weight. Serum ALT and AST were measured by kinetic method. Liver GSH was measured by using colorimetric kits. It can be concluded that administration of D-GalN/LPS caused rat liver injury that was through excess ROS generation. Vitamin E succeeded in ameliorating the deleterious effects of D-GalN/LPS induced oxidative stress.

INTRODUCTION

Acute hepatic failure (AHF) which sometimes called fulminant hepatic failure (FHF) is a sever liver disease which leads to encephalopathy and coagulopathy (Zou et al., 2013). Administration of d-galactosamine (D-GalN) and lipopolysaccharide (LPS) lead to formation of ALF in rats (Jung et al., 2013). D-GalN is very toxic to liver which causes liver injury resembling viral hepatitis with inflammation, degeneration and necrosis. D-GalN alters liver functions through depletion of uridine pools and so, limiting synthesis of RNA and protein (Padmanabhan and Jangle 2014). LPS is the main component of outer gram negative bacteria outer memebrane. Small amount of LPS released from infecting pathogen induce potent innate immune response and led to fatal septic shock syndrome (Zou et al., 2013). LPS bind to LPS binding protein (LBP) in serum which transfer LPS to cluster of differentiation 14 (CD14) either in soluble form or linked by a glycosylphoshatidylinositol anchor of cell surface (Park and Lee 2013). LPS aggregates converted to monomeric molecules and presented to toll like receptor4-MD-2 (TLR4-MD-2) complex by action of CD14 (Cohen 2002; Raetz and Whitfield 2002; Akira et al., 2004).

Co-adminstration of D-GalN and LPS lead to excessive generation of reactive oxygen species (ROS) which was through their oxidative damage lead to acute hepatitis (Jung et al., 2013; Sheik Abdulazeez and Thiruvengadam 2013). Activation of TLR4 in response to LPS cause production of ROS and increased proinflamatory cytokines expression such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-12 and therefore increased ROS production (Medvedev et al., 2000; O'GRADY et al., 2001; Jung et al., 2013).

Oxidative stress is imbalance between pro-oxidants and antioxidants leading to cellular damage. This imbalance can result from decreased antioxidant capacity, or by an overproduction of ROS from other factors (Ha et

al., 2010). The excessive ROS productionhave a pathogenetic role in liver injuries, leading to almost all clinical and experimental conditions of chronic liver diseases (Zhang et al., 2008). Overproduction of ROS must be promptly removed from the cells by enzymatic antioxidant suchassuperoxide dismutase(SOD), catalase (CAT) and the non-enzymatic antioxidant such as Glutathione (GSH)(Martin and Okolie 2012). Free radicals cause many diseases. They attack biological macromolecules in cells and cause damage to DNA and protien with lipid peroxidation (Kumar et al., 2012). The increased lipid peroxidation as well as the decreased of ROS scavengers are taken as a direct evidence for oxidative stress and are a characteristic finding in a variety of diseases (Khan 2006; Srilaxmi et al., 2010).Reactive oxygen species consisted of superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicle ($^{\cdot}OH$). ROS generation leads to lipid peroxidative degradation of the hepatocellular membrane and as a result release of cytoplasmic enzymes, alanine transaminase (ALT) and aspartate transaminase (AST) into the systemic circulation (Sebai et al., 2010).Increased ROS due to administration of D-GalN and LPS caused depletion of GSH, SOD and CAT, and increased production of malondialdehyde (MDA) due to lipid peroxidation (Zhu et al., 2012; Jung et al., 2013).

Glutathione is an important non-enzymatic antioxidant, which is vital to sustain the normal reduced state of cells and to counteract the damage effects of ROS (Saeed et al., 2012). GSH has a protective role in cells by detoxification reactions of endogenous and exogenous toxins and also scavenges free radicals thereby maintaining the structural integrity of cells and organelle (Kim et al., 2012). GSHdeplation may compromise cellular antioxidant defenses and lead to increase of ROS such as $O_2^{\cdot -}$, $OH^{\cdot}$ and H_2O_2, which further initiate lipid peroxidation in cell membranes, and finally caused tissue damage and cellular death (Mershiba et al., 2013). SOD considered the first line of defense mechanism against ROS and catalyzes dismutation of $O_2^{\cdot -}$to oxygen and H_2O_2(Jiang et al., 2009). CAT enzyme catalysis the decomposition of H_2O_2 and lipid peroxides in cell membrane into oxygen and water, by reduced glutathione as a substrate (Olorunnisola et al., 2011).

Vitamin E is the most important lipophilic antioxidant and free radical scavenger (Nazıroğlu et al., 2004). Vitamin E act as a powerful antioxidants with an excellent ability to scavenger the peroxyl radicals and are able to block the formation of hydroxyl radical generated in non-lipid system, so increase the activity of the antioxidant enzymes. In addition these compounds have been shown to inhibit the lipid peroxidation in liver microsomes(Valentin and Qi 2005; Chow and Chow-Johnson 2013). Vitamin E protects cells against oxidative damage induced by LPS (Nishio et al., 2013).

Therefore the present study aimed to investigate the biochemical mechanisms involved in curative and prophylactic effects of silibinin and vitamin E against D-GalN/LPS induced hepatotoxicity in rats.

Materials and Methods

2.1. Materials

Vitamin E, D-GalN and LPS (serotype E.coli 0111:4) were purchased from Sigma Aldrich Chemical Co., St Louis MO, USA). ALT and AST kits were purchased from Human Co. (Wiesbaden, Germany). GSH colorimetric kits obtained from Biodiagnostic Co. (Giza, Egypt)

2.2. Animals

Thirty adult albino rats weighting 180-230g were used in the present study. They were obtained from the animal house of research institute of ophthalmology (Giza, Egypt). Animals were kept under suitable laboratory conditions for two weeks for adaptation before the onset and to exclude any intercurrent infection. They were maintained in stainless steel cages at normal atmospheric temperature of 27±5c as well as under good ventilation. Animals were fed on the standard commercial diet (ATMID Company, Egypt) and provided with tab water ad libitum (Schönfeld et al., 1993).

2.3. Experimental design

The rats were divided randomly into six groups of ten rats each and treated as follows

Group 1 (control): Comprised of 10 rats that received normal diet and injected with normal saline intraperitoneally in a dose of 10 ml/kg for two weeks (Vimal and Devaki 2004).

Group 2 (D-GalN/LPS): Consisted of 10 rats that received normal diet and exposed to single intraperitoneal injection of D-GalN in a dose of 500 mg/kg, followed by single intraperitoneal injection of LPS at a dose of 50 µg/kg (Wilhelm et al., 2009)

Group 3 (Vitamin E): Composed of 10 rats that received normal diet and were administered Vitamin E in a dose of 400 mg/Kg for two weeks (Dillioglugil et al., 2005). Then they were exposed to single intraperitoneal injection of D-GalN/LPS with the above mentioned doses.

After 18 h of overnight fasting, the rats were sacrificed and the blood samples were collected for separation of serum which was kept at -20°C for determination of ALT and AST. Livers were homogenized in 5 ml phosphate buffered saline by using tissue homogenizer (Yellow line DI 18 basic, IKA, Germany). The liver homogenates were centrifuged at 10,000 rpm for 15 minutes. The supernatants were collected and used directly for measurement of GSH.

All animal procedures were performed upon approval from the Ethics committee of Beni-Suef University and in accordance with the recommendations of the proper care and use of lab animals.

2.4. Biochemical analysis

Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were measured by kinetic methods (Human, Wiesbaden, Germany). Reduced GSH levels wasmeasured using colorimetric kits (Biodiagnostic, Giza, Egypt).

2.5. Statistical analysis

All experiments were performed in six groups of ten rats each. The results were expressed as mean ± standard error of mean (SEM). The statistical analysis was performed using one way analysis of variance (ANOVA), followed with Tukey multiple comparison Post-Hoc test. The p-values less than 0.05 ($p<0.05$) were considered significant. All calculations were made using SPSS 22 (SPSS, Chicago, USA). Data were graphed by using Graphpad prism 6 (Graphpad software, Inc., USA).

Results

2.6. Serum markers of hepatic injury

ALT and AST activities were significantly increased in D-GalN/LPS treated group when compared to those of the normal control group as shown in table (1). prophylaxiswith vitamin E significantly reduced ALT and AST elevation as showed in table (1) and Fig. (1).

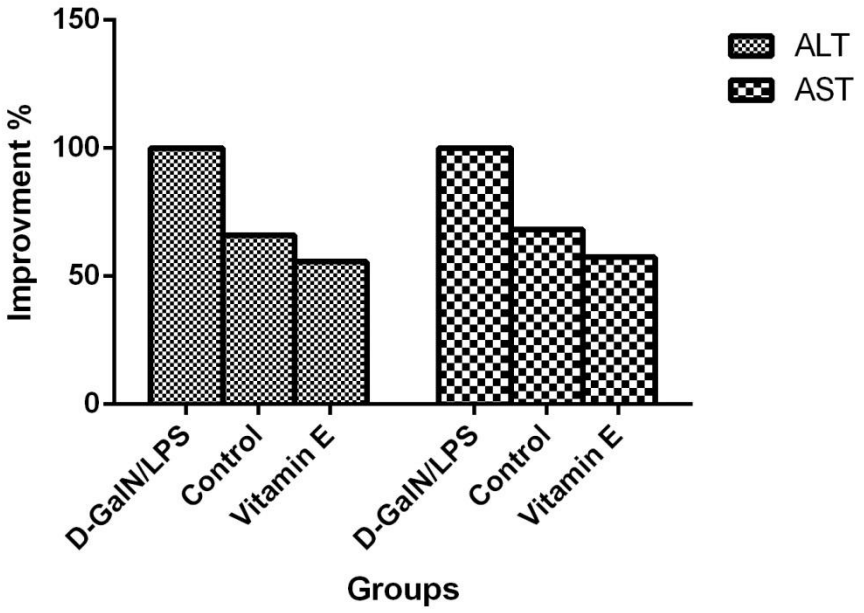

Fig. (1): The percentage of improvement of serum ALT and AST activities in groups received vitamin E compared to D-GalN/LPS treated group.

2.7. Glutathione content

Reduced GSH content of liver homogenate was significantly decreased in D-GalN/LPS treated group when compared to that of control group. prophylaxiswithvitamin E succeeded in improvement of GSH content of liver homogenate as showed in table (1).

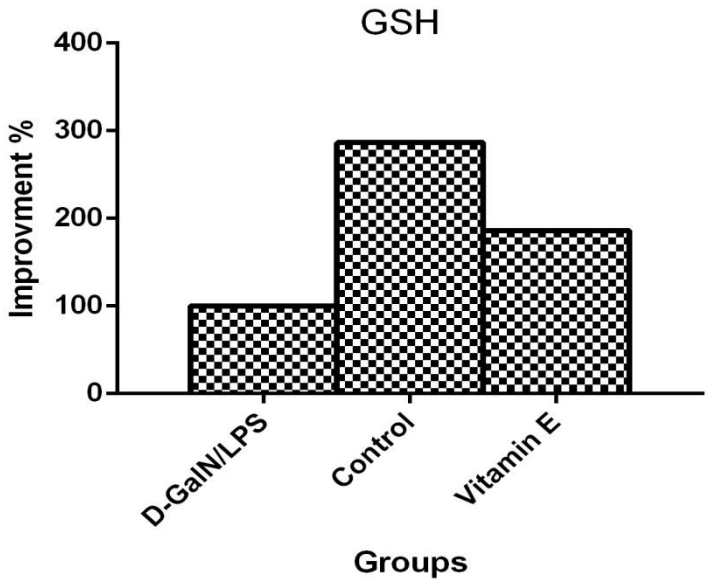

Fig. (2): The percentage of improvement of serum MDA and GSH levels in groups received vitamin E compared to D-GalN/LPS treated group.

Table (1): Serum ALT and AST levels and liver GSH level in the rats of various studied groups

Groups / Parameters	Control	D-GalN/LPS	Vitamin E
ALT (U/L)	31.2±1.5	91.8±2.3[a]	40.7±2.5[b]
AST (U/L)	60.4±1.6	190.2±16.2[a]	81 ±9[b]
GSH (mg/g)	193.4±9.8	72±2.9[a]	134±4[b]

- Values are means ± SEM
- [a] $p < 0.05$ compared with control group.
- [b] $p < 0.05$ compared with D-GalN/LPS treated group

Discussion

Administration of D-GalN and LPS lead to excessive generation of ROS and oxidative damage that lead to acute hepatitis through activation of TLR4 which leads to production of proinflamatory cytokine expression as TNF-α, interleukin-6 (IL-6), IL-12 (Jung et al., 2013; Sheik Abdulazeez and Thiruvengadam 2013).

During D-GalN/LPS induced hepatotoxicity, there are some enzymes can be used as biochemical indicators of liver disorders that response to therapy; like ALT, AST which are indicators of hepatotoxicity, since they are related to the function of the hepatic cells(Farghali et al., 2009; Uslusoy et al., 2011). Alterations in the activities of these enzymes may be due to cellular impairment and dysfunction, thus analysis of these marker enzymes reflects mechanisms of cellular damage and subsequent release of protein, their extracellular turnover and also reflect mechanisms of neoplastic process (Mershiba et al., 2013).

The present study demonstrated that the activities of ALT and AST in the plasma of D-GalN/LPS treated group were significantly increased compared to normal control group. These data were in harmony with the previous study (Farghali et al., 2009) which reported that a significant increase in ALT and AST activities after D-GalN/LPS treated animals compared to control animals. This increase is due to the production of ROS, which led to lipid peroxidative degradation of the hepatocellular membrane and as a result, these cytoplasmic enzymes are released into the systemic circulation. The prophylactic action of vitamin E caused a significant decreased in serum activity of ALT and AST, which elevated as a result of hepatotoxic activity of D-GalN/LPS. These data are parallel line with previous study (Onyema et al., 2006) ,which reported that vitamin E attenuated hepatotoxicity and oxidative stress in rats and decreased the elevated activity of ALT and AST as result of liver damage.

Data presented in this study indicated that D-GalN/LPSadministration resulted in augmentation of oxidative stress in livers of treated rats accompanied by impaired antioxidative defense mechanism, as indicated by a

significant depletion of free radical scavenging antioxidants as reduced GSH compared to normal control group. The observations of current study are in accordance with previous studies (Wilhelm et al., 2009; Lekic et al., 2011) which reported that there is a significant decreased in the level of GSH in D-GalN/LPS treated rats compared to the control animals. This might be attributed to the reduction in their biosynthesis during hepatocellular damage or their excessive utilization in scavenging the free radicals formed due to D-GalN/LPS toxicity. In the same regard many studies (Srilaxmi et al., 2010; Huang et al., 2012) reported that the decreased concentration of GSH in liver may be due to NADPH reduction or GSH utilization in the exclusion of peroxides.

Enhancing endogenous enzymatic and non-enzymatic antioxidant status by the hepatoprotective compounds represent an effective strategy in the prevention of hepatic disorders (de Araujo Ribeiro et al., 2011). In this study the supplementation of vitamin E to D-GalN/LPS treated animals effectively modulate the deterioration the antioxidant parameters as GSH.

The present study also, revealed that vitamin E groups were significantly increased the level of GSH in the treated group compared to that of D-GalN/LPSadministered group. The current results are in parallel line with previous studies (Ganesh et al., ; Ganesh et al., 2012; Chow and Chow-Johnson 2013) which reported that this hepatoprotective effect of vitamin E could be attributed to its ability to scavenge the free radicals that induce lipid peroxidation. Vitamin E was foundto dose dependently decrease rates of mitochondrial hydrogen peroxide generation in liver and skeletal muscle through its ability to scavenge the free radicals and modulation of cellular antioxidant.

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