

Protective Effect of Thymoquinone against D-Galactosamine-Induced Liver Injury in Rats

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Abstract: The present study was conducted to investigate the effect of thymoquinone (TQ), a natural main constituent of *Nigella sativa* seeds, in modulation of the altered hepatic function in experimental model of liver injury induced by D-galactosamine (D-GaIN) in an attempt to identify its potential mechanism(s) of action. Rats were divided into four groups: normal control group, D-GaIN control group receiving single i.p. injection of D-galactosamine (400 mg/kg b.w.), TQ-D-GaIN-treated group receiving thymoquinone (20 mg/kg b.w., daily for 10 days) by gavage, prior to D-GaIN injection and silymarin-D-GaIN-treated group receiving silymarin (25 mg/kg b.w., daily for 10 days) by gavage prior to D-GaIN injection. Rats were sacrificed 22 hours following the last injection and subjected to measurement of plasma levels of total proteins, albumin, alanine transferase (ALT), aspartate transferase (AST) and lactate dehydrogenase (LDH), blood levels of reduced glutathione (GSH) and hepatic levels of GSH and malondialdehyde (MDA). Also, liver tissue histopathology was evaluated. TQ pretreatment resulted in marked reduction in the elevated plasma ALT, AST and LDH and hepatic MDA levels induced by D-GaIN intoxication, which was comparable to that produced by silymarin. However, there was a slight rise in the declined plasma total proteins and albumin and hepatic GSH caused by D-GaIN, in rats pretreated with TQ, in contrast to the significant rise in these parameters with silymarin. Moreover, histopathological examination of the liver sections confirmed that the normal liver architecture was damaged with D-GaIN injection. However, pretreatment with TQ significantly decreased the severity of histopathological injury, nearly resembling silymarin. In conclusion, pretreatment with TQ protected the liver enzyme leakage and prevented lipid peroxidation induced by D-GaIN, indicating that the membrane stabilizing effect of TQ might be ascribed to its ability to scavenge the free radicals produced by D-GaIN and therefore protects the liver cell against oxidative damage.

Key words: D-galactosamine, thymoquinone, hepatoprotective effect and lipid peroxidation.

INTRODUCTION

The liver, the largest gland in the body, has many complex functions essential for life. Therefore, systemic abnormalities often occur in patients with liver dysfunction (Minemura *et al.*, 2009). Hepatic injury is complicating some diseases as diabetes mellitus (El-Serag and Everhart, 2002) or manifested as side effects of some medications (Landolina *et al.*, 1984). Acute liver injury may cause dismal clinical outcome (Shakil *et al.*, 2000 and Logurecio and Federico, 2003); however, the detailed pathophysiological mechanisms and the preventive and therapeutic medications have not been elucidated, giving liver protection special concern.

D-galactosamine (D-GaIN) is known for inducing the features of acute hepatitis in rats (Ferenciková *et al.*, 2003) and is regarded as a transcriptional inhibitor (Leist *et al.*, 1994). D-GaIN-induced liver damage is similar to human viral hepatitis in its morphological and functional features (Keppler *et al.*, 1968).

Thymoquinone (TQ), the active ingredient of *Nigella sativa*, was found to exert antioxidative (Nagi and Mansour, 2000) and anti-inflammatory (Houghton *et al.*, 1995) functions. In addition, a number of studies indicated the hepatoprotective effect of TQ against tert-butyl hydroperoxide toxicity in isolated rat hepatocytes

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(Daba and Abdel-Rahman, 1998), and carbon tetrachloride-induced hepatotoxicity in mice (Mansour, 2000), as well as in experimental models of epilepsy in mice (Raza *et al.*, 2006) and ethanol-induced hepatotoxicity in rats (Alsaif, 2007).

It was, therefore, interesting to investigate the possible favorable potential of TQ on the D-GaIN-induced liver dysfunction in rats. The results were compared with silymarin, a well documented hepatoprotective drug, in an attempt to evaluate the magnitude of protection offered by TQ and to assess its use as a promising prophylactic agent against hepatic injury.

MATERIALS AND METHODS

Experimental Animals:

The present study was performed on 44 adult female Wistar rats, weighing 180-210 g. Rats were purchased from the Research Institute of Ophthalmology (Giza, Egypt), and maintained in the Physiology Department Animal House under standard conditions of boarding. Rats were given regular diet composed of bread, milk and vegetables with free access to water. The care of animals and all experimental procedures were approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University, Egypt (FMASU REC).

Experimental Design:

Rats included in the present study were randomly allocated into four groups:

Normal control group (n= 11): Rats received olive oil by gavage in a dose of 2 ml/kg b.w. daily, for 10 days. On the tenth day, 2 hours after olive oil administration, rats received a single i.p. injection of normal saline in a dose of 1.5 ml/kg b.w. D-galactosamine control group (n= 11): Rats received olive oil by gavage in a dose of 2 ml/kg b.w. daily, for 10 days. On the tenth day, 2 hours after olive oil administration, rats received a single i.p. injection of D-GaIN (Sigma, USA) in a dose of 400 mg in 1.5 ml normal saline per kg b.w. (Lin *et al.*, 2009).

Thymoquinone-D-galactosamine-treated group (n=11): Rats received TQ (Sigma, USA), in a dose of 20 mg in 2 ml olive oil per kg b.w. (Attia *et al.*, 2010), by gavage, daily, for 10 days. On the tenth day, 2 hours after olive oil administration, rats received a single i.p. injection of D-GaIN in a dose of 400 mg in 1.5 ml normal saline per kg b.w. Silymarin-D-Galactosamine-treated group (n= 11): Rats received silymarin (Sigma, USA) in a dose of 25 mg in 2 ml olive oil per kg b.w. (Lai *et al.*, 2010), by gavage, daily, for 10 days. On the tenth day, 2 hours after olive oil administration, rats received a single i.p. injection of D-GaIN in a dose of 400 mg in 1.5 ml normal saline per kg b.w. All animals were sacrificed after 22 hours from the last injection.

Experimental Procedures:

On the day of experiment, overnight fasted rats, with free access to water, were weighed and anaesthetized by i.p. injection of thiopental sodium (EPICO, Egypt), in a dose of 40 mg /kg. A midline abdominal incision was made, and the abdominal aorta was exposed and blood samples were collected in heparinized plastic tubes.

0.1 ml of blood was separated for immediate measurement of GSH in blood. The rest of the blood sample was centrifuged at 3000 rpm for 15 min to separate plasma which was used for determination of ALT, AST, LDH, total proteins and albumin.

The liver was dissected and the right lobe was washed in ice cold saline, and stored at -80°C until used for determination of hepatic GSH and MDA contents. The left lobe was divided into 2 parts, one part was fixed in 10% formalin for light microscopy and the other was fixed in 2.5% glutaraldehyde for electron microscopic study.

Biochemical Analysis:

Total proteins, albumin, ALT, AST and LDH were measured in plasma by colorimetric methods, using kits supplied by Biolabo SA, France. Blood GSH level was determined by colorimetric method, using kits supplied by Bio-diagnostic, Egypt. Assays were performed according to the manufacturer's instructions.

The right lobe of the liver was divided into two pieces. One piece of liver tissue was homogenized in cold buffer (50 mM potassium phosphate, pH 7.5, and 1 mM EDTA) and the other piece was homogenized in another cold buffer (50 mM potassium phosphate, pH 7.5) to prepare the homogenate used for determination of GSH and MDA, respectively, using tissue homogenizer (IKA-WERK, Ultra-Turrax, West Germany). The homogenate was then centrifuged at 4000 rpm for 15 min and the supernatants were stored at -80°C until used for determination of GSH and MDA using kits supplied by Bio-diagnostic, Egypt.

Histopathological Study:

- 1- Light microscopic study: Small liver specimens were fixed in 10% neutral formalin and processed to get paraffin sections of 5 µm thickness. Sections were stained with Hematoxylin and Eosin (H&E) (Bancroft and Gamble, 2008). Examination of the H&E sections was done using the light microscope for evaluation of degeneration and increase in eosinophilia of hepatocytes and inflammatory cell infiltration. Grading and scoring was conducted as the following: 0 score = no observed changes, 1 point score = minimal changes, 2 points score = mild changes, 3 points score = moderate changes and 4 points score = severe changes (Willis and Asha, 2006).
- 2- Electron microscopic study: The liver specimens were cut in small pieces (1mm³) and fixed in gluteraldehyde. Specimens were washed in 0.1 M phosphate buffer at 4°C, then post fixed in 1% osmium tetroxide. Specimens were dehydrated, then embedded in Epon resin. Ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate. Specimens were examined and photographed with JEM 1200 EXII transmission electron microscope (TEM) in Faculty of Science, Ain Shams University (Bancroft and Gamble, 2008).

Statistical Analysis:

The data were expressed as mean ± SEM. All statistical comparisons were made by mean of a one-way ANOVA test followed by LSD (least significant difference) multiple range-test to find inter-groups' significance. Correlations and lines of regression were calculated by linear regression analysis using the Least Square Method. All analyses were made using SPSS statistical software package (SPSS Inc.) version 16.0.1. A probability of P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results:

Effects of TQ on Plasma Proteins, Albumin, ALT, AST and LDH in D-GalN Intoxicated Rats:

Rats receiving a single injection of D-GalN (400 mg/kg b.w.) showed a significant reduction in plasma total proteins (P<0.005) and albumin (P<0.05), as compared to normal control group, whereas rats treated with thymoquinone (20 mg/kg b.w.) prior to D-GalN injection displayed a non significant increase in these parameters, compared to D-GalN control group. However, pretreatment with silymarin affirmed a significant rise in plasma levels of total proteins and albumin (P<0.002, and P<0.001, respectively) compared to that in rats injected with D-GalN, to become comparable to normal control values (table 1).

Table 1: Mean ± SEM of plasma levels of total proteins, albumin, alanine transferase (ALT), aspartate transferase (AST), and lactate dehydrogenase (LDH), as well as, blood levels of reduced glutathione (GSH) and hepatic contents of GSH and malondialdehyde (MDA), in the different studied groups, compared by ANOVA.

Parameters	Normal control	D-GalN control	Thymoquinone-D-GalN	Silymarin-D-GalN
Total proteins (g/dl)	6.18±0.22 (11)	5.26±0.19 ^a (11)	5.75±0.19 (11)	6.25±0.17 ^b (11)
Albumin (g/dl)	2.96±0.15 (11)	2.46±0.15 ^a (11)	2.80±0.19 ^c (11)	3.35±0.09 ^b (11)
ALT (IU/L)	46.35±15.16 (11)	660.36±213.32 ^a (11)	107.10±23.66 ^b (11)	115.62±26.12 ^b (11)
AST (IU/L)	64.88±5.89 (11)	633.19±234.43 ^a (11)	149.59±30.19 ^b (11)	191.11±28.78 ^b (11)
LDH (IU/L)	237.19±19.43 (11)	1032.80±214.36 ^a (11)	407.86±45.56 ^b (11)	363.23±66.53 ^b (11)
Blood GSH (mg/dl)	26.57±2.79 (11)	27.30±1.78 (10)	26.33±0.97 (9)	27.50±1.43 (11)
Hepatic GSH (mg/g)	55.47±4.55 (11)	37.25±7.82 (11)	38.26±2.60 ^c (11)	60.84±8.71 ^b (11)
Hepatic MDA (nmol/g)	30.58±3.75 (11)	67.42±5.45 ^a (11)	43.85±4.59 ^b (11)	42.90±7.14 ^b (11)

(a): Significant difference from normal control group, by LSD at least P< 0.05 (b): Significant difference from D-galactosamine control group, by LSD at least P< 0.05 (c): Significant difference from silymarin-D-galactosamine-treated group, by LSD at least P< 0.05 Values in parenthesis indicate number of rats in each group. D-GalN: D-Galactosamine

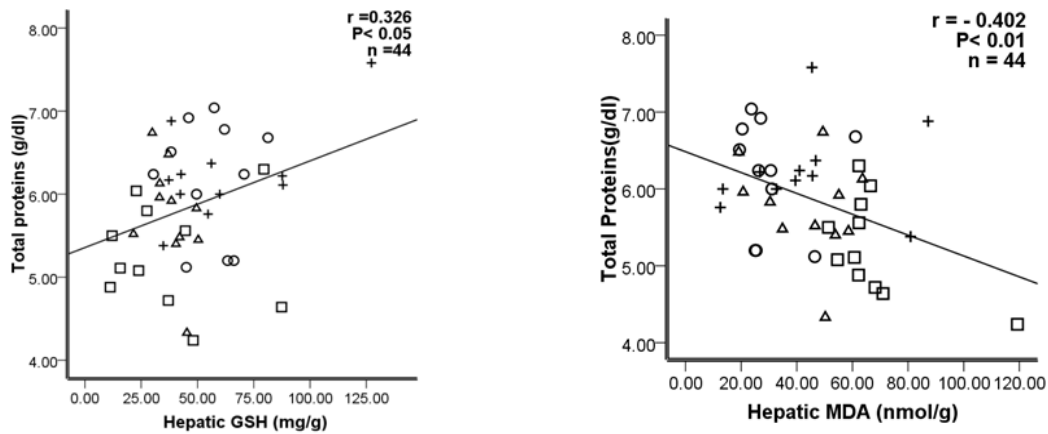


Fig. 1: Plasma total proteins were positively correlated with hepatic reduced glutathione (GSH) and negatively correlated with hepatic malondialdehyde (MDA), in 44 rats from the four studied groups; Normal control group (○), D-galactosamine control group (□), Thymoquinone-D-galactosamine-treated group (Δ) and Silymarin-D-galactosamine-treated group (+).

On the other hand, plasma levels of ALT, AST, and LDH were significantly elevated in rats injected with D-GaIN ($P<0.001$, $P<0.005$, $P<0.001$, respectively) compared to normal control rats. Pretreatment with TQ, resulted in a significant drop in the elevated ALT, AST, and LDH ($P<0.002$, $P<0.01$, and $P<0.001$, respectively), as compared to the corresponding values in the D-GaIN control group, however, these parameters were still non significantly higher than that in normal control rats. Similarly, pretreatment with silymarin significantly decreased the elevated plasma levels of ALT, AST, and LDH, induced by D-galactosamine injection (table 1).

Effects of TQ on GSH and MDA in D-GaIN Intoxicated Rats:

Data obtained in table (1) reveal a significant increment of MDA content ($P<0.001$), and a non significant reduction of GSH content in the liver homogenate of D-GaIN control group as compared to that of their respective normal control rats. Pretreatment with TQ induced a remarkable protection against peroxidative damage caused by D-GaIN intoxication, as MDA content in liver homogenate was significantly decreased ($P<0.005$), compared to D-GaIN control group, and non significantly changed compared to normal control group, in addition to being comparable to hepatic MDA content in silymarin-D-GaIN treated group. Furthermore, TQ-D-GaIN-treated group provoked non significant changes in GSH in both blood and liver homogenate, compared to the other three groups, except for significant reduction in hepatic GSH content ($P<0.02$), compared to silymarin. Pretreatment with silymarin induced a significant fall in hepatic MDA content ($P<0.005$), in contrast to a significant rise in hepatic GSH content ($P<0.02$), and non significant changes in blood GSH level, compared to D-GaIN control group, but all these parameters were non significantly different from that in the normal control group.

In fig. 1, plasma total proteins showed a significant negative correlation with hepatic MDA ($r=-0.402$, $P<0.01$, $n=44$) and a significant positive correlation with hepatic GSH ($r=0.326$, $P<0.05$, $n=44$).

Table 2: Mean \pm SEM of scores of histopathological changes in rat livers, stained with hematoxylin and eosin, in the different studied groups, compared by ANOVA.

Parameters	Normal control	D-GaIN control	Thymoquinone-D-GaIN	Silymarin-D-GaIN
Degeneration and necrosis of hepatocytes	0.00 \pm 0.00	3.09 \pm 0.83 ^a	2.27 \pm 1.01 ^{ab}	2.18 \pm 0.75 ^{ab}
Increase eosinophilia of hepatocytes	0.00 \pm 0.00	2.73 \pm 0.79 ^a	1.73 \pm 0.79 ^{ab}	1.46 \pm 0.52 ^{ab}
Inflammatory cell infiltration	0.00 \pm 0.00	3.00 \pm 0.78 ^a	1.82 \pm 0.75 ^{ab}	1.55 \pm 0.93 ^{ab}

(a): Significant difference from normal control group, by LSD at least $P<0.05$

(b): Significant difference from D-galactosamine control group, by LSD at least $P<0.05$

Livers of 11 rats in each group were examined.

D-GaIN: D-Galactosamine

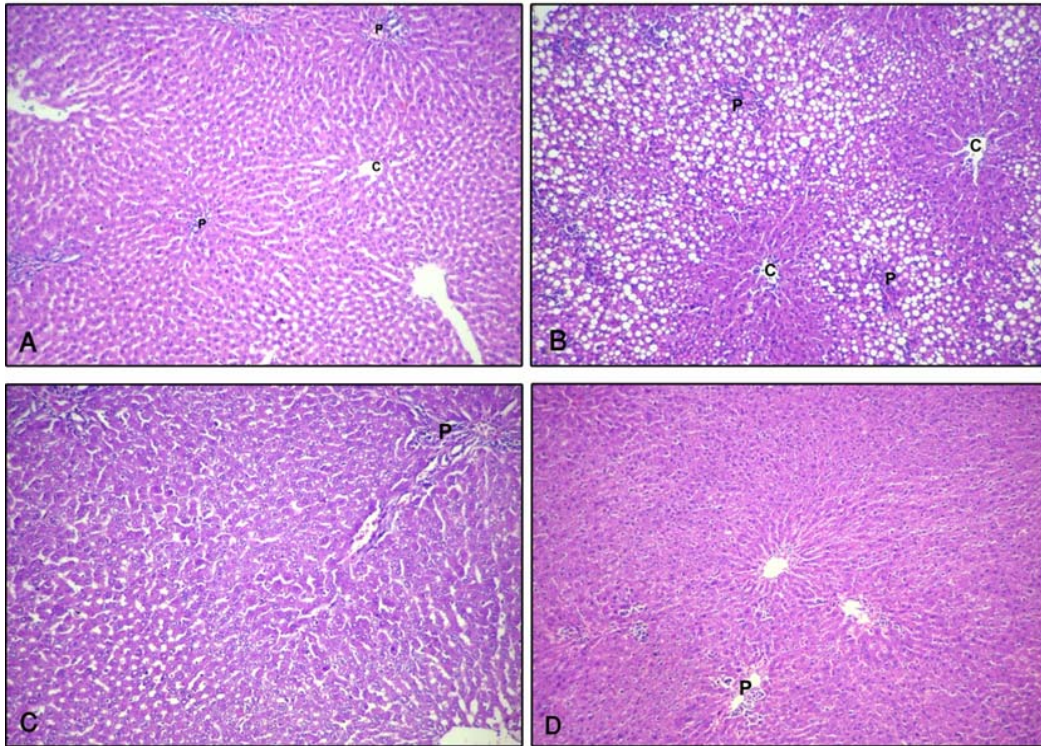


Fig. 2: Photomicrographs of rats liver sections of: (A) control group: showing normal hepatic lobule with central vein “C” and peripherally-situated portal areas “P”. (B) D-galactosamine control group: showing vacuolated hepatocytes surrounding portal areas “P”. The cells near to the central veins “C” show less vacuoles with increased eosinophilia. Notice increased inflammatory cell infiltration. (C) thymoquinone-D-galactosamine-treated group: showing decrease hepatocytes affection and inflammatory cell infiltration in the portal area “P” than D-galactosamine group. (D) silymarin-D-galactosamine-treated group: showing minimal hepatocytes affection with less inflammatory cell infiltration in the portal area “P” compared to D-galactosamine and thymoquinone-D-galactosamine-treated groups. (H&E X 200)

Effects of TQ on Liver Histopathology in D-GaIN Intoxicated Rats:

Examination of the H&E stained section of the D-GaIN control group showed focal and bridging hepatic necrosis. Vacuolated and degenerated hepatocytes appeared surrounding portal areas and extending towards central veins ($P < 0.001$) with increase eosinophilia of hepatocytes ($P < 0.001$). Further, inflammatory cell infiltration were observed around the central vein, in the portal areas and in blood sinusoids ($P < 0.001$), as compared to the normal control (fig. 2B, table 2). The liver sections of the D-GaIN group examined by TEM showed many lipid droplets of different sizes compressing the nuclei and causing irregularity of their nuclear membrane. The cytoplasm showed fewer rough endoplasmic reticulum (rER) and glycogen rosettes in-between high electron-dense mitochondria and dilated smooth endoplasmic reticulum (sER), compared to the control group (fig. 3B).

Pretreatment with TQ and silymarin showed a noticeable improvement in D-GaIN-induced liver damage as there were fewer lipid droplets, less electron-density of mitochondria and less dilated sER in TEM examination (figs. 3C, and 3D), compared to D-GaIN group. Also, figs. 2C, and 2D and table 2 reveal that the preadministration of TQ and silymarin resulted in a significant reduction in vacuolar degeneration and necrosis ($P < 0.02$ and $P < 0.01$, respectively), eosinophilia of hepatocytes ($P < 0.001$) and the inflammatory cell infiltrates ($P < 0.001$), as compared to the D-GaIN group. But, no significant differences were found between TQ-D-GaIN-treated and silymarin-D-GaIN-treated groups.

Discussion:

In this study, D-GaIN injected rats revealed elevated plasma levels of liver enzymes; ALT, AST and LDH, and as well decreased plasma total proteins and albumin, diminished hepatic GSH content and raised hepatic

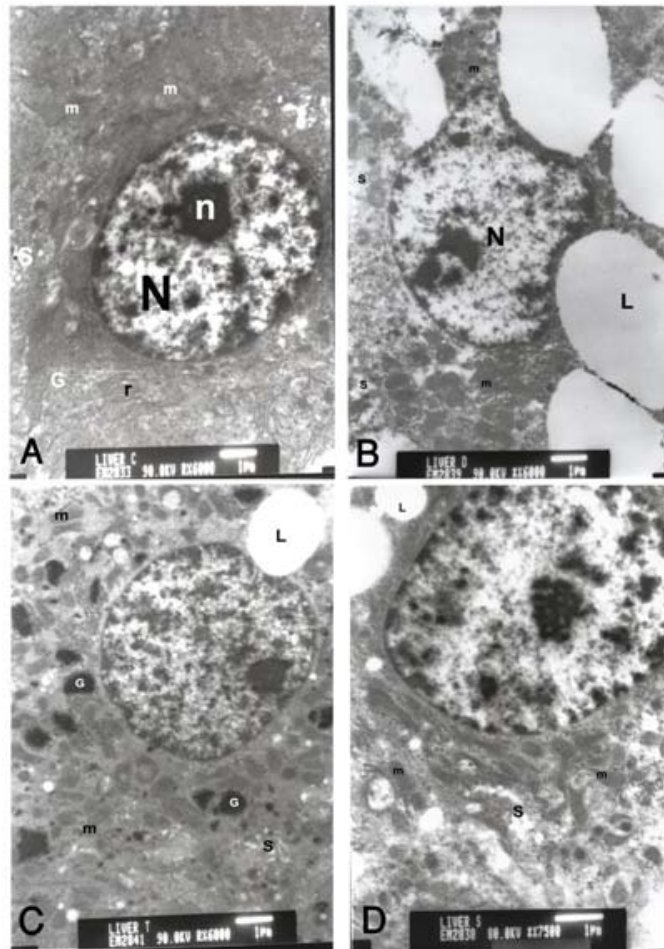


Fig. 3: Electron micrographs of rats liver sections of: (A) normal control group: showing a hepatocyte with rounded nucleus “N” and prominent nucleolus “n”, many mitochondria (m), rER “r”, scattered glycogen rosettes “G” and some sER “S”. (B) D-galactosamine control group: showing the nucleus “N” compressed by large lipid droplets “L”. The cytoplasm shows fewer rER and glycogen rosettes in-between high electron-dense mitochondria “m” and dilated sER “S” compared to the control group. (C) thymoquinone-D-galactosamine- treated group: showing less lipid droplets “L”, less electron-density of mitochondria “m”, less dilated sER “S” and condensed glycogen rosettes “G” compared to D-galactosamine group. (TEM X 6000). (D) silymarin-D-galactosamine-treated group: showing fewer lipid droplets “L” and mild electron-dense mitochondria “m” compared to D-galactosamine group. Notice some dilated sER “S”. (TEM X 7500).

MDA content. Upon TQ pretreatment (20 mg/kg b.w.) for 10 days prior to D-GaIN administration, marked reduction was observed in the elevated plasma levels of liver enzymes and hepatic MDA content, whereas plasma total proteins and albumin showed a slight increase. Although TQ prevented D-GaIN-induced increase in levels of plasma ALT, AST and LDH and hepatic MDA to the same extent as silymarin, the protective effect offered by TQ on plasma albumin level and hepatic GSH content was less than that by silymarin.

D-GaIN is a well-established hepatotoxicant that induces a diffuse type of liver injury morphologically and functionally closely resembling human viral hepatitis (Decker and Keppler, 1972). In the present study, the marked elevation in plasma levels of ALT, AST and LDH induced by D-GaIN is consistent with a number of earlier reports (Muntané *et al.*, 2000; Wills and Asha, 2006 and Zhou *et al.*, 2008) and could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage (Naik and Panda, 2007).

D-GaIN predominantly inhibits hepatic glucuronidation since it markedly depletes hepatic uridyl diphosphate glucuronic acid (UDP-GA), whereas extrahepatic UDP-GA is minimally affected. This disrupts

the synthesis of essential uridylyate nucleotides resulting in organelle injury. Depletion of uracil nucleotides in hepatocytes ultimately impairs the synthesis of protein and glycoprotein, and thus leads to progressive damage of cellular membranes, resulting in a change in permeability of the cellular membrane which leads to enzyme leakage from the cells (Keppler *et al.*, 1970 and Abdul-Hussain and Mehendale, 1991). Collecting the above data, this elevation in plasma ALT, AST and LDH encountered, herein, with D-GaIN administration could be a result of liver cell destruction and/or a change in membrane permeability.

On the other hand, the increased plasma LDH obtained, herein with D-GaIN administration, indicates necrosis of hepatocytes, as demonstrated earlier in hepatocyte culture medium (Quintero *et al.*, 2002). In support, our histopathological observations confirmed that the normal liver architecture was damaged with D-GaIN administration characterized by vacuolated and degenerated hepatocytes, surrounding portal areas and extending towards central veins with appearance of focal and bridging hepatic necrosis.

Pretreatment with TQ at a dose of 20 mg/kg, daily, for 10 days, significantly suppressed the elevated levels of plasma ALT, AST, and LDH induced by D-GaIN which was in close similarity with that by silymarin. Our results come in concert with earlier reports which indicated the hepatoprotective effect of TQ in models of liver injury in vitro (Daba and Abdel-Rahman, 1998), and in vivo (Raza *et al.*, 2006 and Alsaif, 2007). The efficacy of TQ in protecting hepatic enzyme leakage is related to its ability to preserve the structural and functional integrity of the liver against the adverse effects of D-GaIN.

In addition, the affection of synthetic function of the liver cells in D-GaIN group is shown by the significant decline in plasma total proteins and albumin. The reduction of serum albumin could be attributed to the reduction of albumin mRNA expression. Dabeva and Shafritz (1993) found that mRNA for albumin, the most abundantly expressed liver specific gene, decreases abruptly after D-GaIN treatment.

Although TQ pretreatment provoked some elevation in the reduced plasma levels of total proteins and albumin after D-GaIN intoxication, the elevation was less than that caused by silymarin, in which levels of these parameters were comparable to normal controls. This tendency to increase the levels of plasma total proteins and albumin could be ascribed to suppression of liver damage induced by D-GaIN with some improvement of liver synthetic function following TQ treatment.

MDA is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (Vaca *et al.*, 1988). MDA, a secondary product of lipid peroxidation, is a useful indicator of tissue damage involving a series of chain reactions (Ohkawa *et al.*, 1979). The increased hepatic MDA with D-GaIN observed in the present study is a convenient marker of enhanced lipid peroxidation. Zhou *et al.* (2008) ascribed D-GaIN -induced liver damage and consequently, liver dysfunction to promotion of oxidative stress. Previously, Quintero *et al.* (2002) reported that the mitochondrial dysfunction is a potential main source of intracellular free radicals production during the induction of cell death. In concert, our electron microscopic findings indicated the presence of affected electron-dense mitochondria, few rough endoplasmic reticulum, dilatation of smooth endoplasmic reticulum, and damage of the nucleoli of rat hepatocytes in D-GaIN group. These results support implication of increased free radicals in D-GaIN - induced liver damage.

Treatment of rats with TQ protected the liver from increased hepatic MDA induced by D-GaIN, which was comparable to that encountered with silymarin. This decline in hepatic MDA demonstrates the antilipid peroxidative effect of TQ, denoting its free radical scavenging activity. It has been reported that TQ inhibits non-enzymatic lipid peroxidation in liposomes (Houghton *et al.*, 1995) and in liver homogenate (Nagi *et al.*, 1999) and has appreciable free radical scavenging properties (Burtis and Bucar, 2000). This antioxidant property would partly explain its action against hepatotoxicity (Ilhan and Seçkin, 2004).

Histopathological observations, in the present study, supported the membrane stabilizing effect of TQ in D-GaIN administered rats. Focal and bridging necrosis, increased eosinophilia of hepatocytes and inflammatory cell infiltration induced by D-GaIN were significantly lessened by pretreatment with TQ. However, more protection was observed with silymarin, yet it was non significantly different compared to TQ.

GSH is found in a particularly high concentration in the liver and has key functions in protective processes. It has been demonstrated that lipid peroxidation is concurrent with GSH depletion (Seçkin *et al.*, 1993). In our study, D-GaIN-induced hepatotoxicity was associated with a prominent decrease in the hepatic GSH content, in agreement with previous studies, suggesting suppression of hepatic GSH by the oxidative stress induced by D-GaIN (Pushpavalli *et al.*, 2008 and Ying-Wan *et al.*, 2010).

Furthermore, plasma total proteins were positively correlated with hepatic GSH content and negatively correlated with hepatic MDA content. This gives support for the inhibition of the synthetic function of the liver, evident by the decrease in plasma proteins and albumin in concurrence with the decrease in GSH content and the increase in hepatic MDA content observed herein following D-GaIN injection, confirming liver injury in this context.

It is worth mentioning that the significant increase in hepatic MDA and the prominent decrease in hepatic GSH content, observed in the current study in D-GaIN intoxicated rats, point to a disequilibrium between the oxidant and antioxidant balance suggesting that liver injury-induced by D-GaIN could be ascribed to enhancing the oxidative stress and/or decreasing the antioxidant defense.

Interestingly, the effect of TQ on hepatic GSH content is controversial. In the present study, TQ treatment at a dose of 20 mg/kg b.w. did not prevent the decline in hepatic GSH content induced by D-GaIN, in contrast to silymarin that significantly increased it to be comparable to normal control value. Recently, Nagi and Almakki (2009) reported a marked depletion of hepatic GSH with TQ administration in normal mice, while Helal (2010) and Nagi *et al.* (2010) demonstrated an increase in hepatic GSH associated with TQ protective effect against experimentally induced hepatic injury.

The inability of TQ to increase the liver GSH content, contrary to the results of silymarin, could be explained by the ability of TQ to react with GSH, producing glutathione conjugates *in vitro* (Wang *et al.*, 2006 and Chan *et al.*, 2008). Also, TQ was found to react with GSH through spontaneous and rapid reaction that forms glutathionyl-dihydrothymoquinone which has a higher scavenging activity and is considered as an important lipid soluble antioxidant (Khalife and Lupidi, 2007). Thus, these reactions of TQ, although partially decreasing the GSH content in the cells, they magnify the antioxidant activity of TQ (Khalife and Lupidi, 2007). Indeed, further studies with higher doses of TQ are required to clarify its exact effect on hepatic GSH contents and antioxidant enzyme levels.

In conclusion, pretreatment with TQ protected the liver enzyme leakage and prevented lipid peroxidation induced by D-GaIN to the same extent as silymarin, indicating that the membrane stabilizing effect of TQ might be ascribed to its ability to scavenge the free radicals derived from D-GaIN and therefore protect the liver from oxidative damage. However, the magnitude of protection offered by TQ was less than that of silymarin. Thus, further studies with higher doses of TQ and for longer duration are necessary to determine the effective dose and to assess its value as a promising prophylactic agent against hepatic dysfunction.

REFERENCES

- Abdul-Hussain, S.K. and H.M. Mehendale, 1991. Studies on the age-dependent effects of galactosamine in primary rat hepatocyte cultures. *Toxicol Appl Pharmacol.*, 107: 504-513.
- Alsaif, M.A., 2007. Effect of thymoquinone on ethanol-induced hepatotoxicity in Wistar rats. *J Med Sci.*, 7: 1164-1170.
- Attia, A., A. Ragheb, T. Sylwestrowicz and A. Shoker, 2010. Attenuation of high cholesterol-induced oxidative stress in rabbit liver by thymoquinone. *Eur J Gastroenterol Hepatol.*, 22: 826-834.
- Bancroft, J.D. and M. Gamble, 2008. *Theory and practice of histological techniques*. 6th ed. Churchill Livingstone Elsevier.
- Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res.*, 14: 323-328.
- Chan, K., N. Jensen and P.J. O'Brien, 2008. Structure-activity relationships for thiol reactivity and rat or human hepatocyte toxicity induced by substituted p-benzoquinone compounds. *J Appl Toxicol.*, 28: 608-620.
- Daba, M.H. and M.S. Abdel-Rahman, 1998. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicol Lett.*, 95: 23-29.
- Dabeva, M.D. and D.A. Shafritz, 1993. Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. *Am J Pathol.*, 143: 1606-1620.
- Decker, K. and D. Keppler, 1972. Galactosamine-induced liver injury. In: *Progress in liver disease*, Eds., Popper, H. and Schaffner, F., vol 4. New York: Grune and Stratton, pp: 183-196. (Cited by Abou-Ellella, A., Siendones, E. Padillo, J., Montero, J.L., M. De la Mata, and Muntané Relat, J., 2002. Tumour necrosis factor- α and nitric oxide mediate apoptosis by D-galactosamine in a primary culture of rat hepatocytes: exacerbation of cell death by cocultured Kupffer cells. *Can J Gastroenterol.*, 16: 791-799.
- El-Serag, H.B. and J.E. Everhart, 2002. Diabetes increases the risk of acute hepatic failure. *Gastroenterology*, 122: 1822-1828.
- Ferenciková, R., Z. Cervinková and Z. Drahotá, 2003. Hepatotoxic effect of D-galactosamine and protective role of lipid emulsion. *Physiol Res.*, 52: 73-78.
- Helal, G.K., 2010. Thymoquinone supplementation ameliorates acute endotoxemia-induced liver dysfunction in rats. *Pak J Pharm Sci.*, 23: 131-137.
- Houghton, P.J., R. Zarka, B. de las Heras and J.R. Houtl, 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.*, 61: 33-36.

- İlhan, N. and D. Seçkin, 2005. Protective effect of *Nigella sativa* seeds on CCL4- induced hepatotoxicity. *F.Ü. Sağlık Bil. Dergisi.*, 19: 175-179.
- Keppler, D., R. Lesch, W. Reutter and K. Decker, 1968. Experimental hepatitis induced by D-galactosamine. *Exp Mol Pathol.*, 9: 279-290.
- Keppler, D.O., J.F. Rudigier, E. Bischoff and K.F. Decker, 1970. The trapping of uridine phosphates by D-galactosamine, D-glucosamine, and 2-deoxy-D-galactose. A study on the mechanism of galactosamine hepatitis. *Eur J Biochem.*, 17: 246-253.
- Khalife, K.H. and G. Lupidi, 2007. Nonenzymatic reduction of thymoquinone in physiological conditions. *Free Radic Res.*, 41: 153-161.
- Lai, T.Y., Y.J. Weng, W.W. Kuo, L.M. Chen, Y.T. Chung, Y.M. Lin, F.J. Tsai, C.H. Lee, Y.M. Choong, E.Y. Lai, C.Y. Huang and Y.L. Yeh, 2010. Taohe Chengqi Tang ameliorates acute liver injury induced by carbon tetrachloride in rats. *Zhong Xi Yi Jie He Xue Bao.*, 8: 49-55.
- Landolina, M., P. Beck Peccoz, G. Piscitelli, A.P. Maggioni, A. Valopi, F. Galleti, G. Tognoni, G. Ferrario and P. Giani, 1984. Side effects during therapy with low dose amiodarone. *G Ital Cardiol.*, 14: 723-726.
- Leist, M., F. Gantner, I. Böhlinger, P.G. Germann, G. Tiegs and A. Wendel, 1994. Murine hepatocyte apoptosis induced in vitro and in vivo by TNF-alpha requires transcriptional arrest. *J Immunol.*, 153: 1778-1788.
- Lin, B.R., C.J. Yu, W.C. Chen, H.S. Lee, H.M. Chang, Y.C. Lee, C.T. Chien and C.F. Chen, 2009. Green tea extract supplement reduces D-galactosamine-induced acute liver injury by inhibition of apoptotic and proinflammatory signaling. *J Biomed Sci.*, 25: 16-35.
- Loguercio, C. and A. Federico, 2003. Oxidative stress in viral and alcoholic hepatitis. *Free Radic Biol Med.*, 34: 1-10.
- Mansour, M.A., 2000. Protective effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. *Sci.*, 66: 2583-2591.
- Minemura, M., K. Tajiri and Y. Shimizu, 2009. Systemic abnormalities in liver disease. *World J Gastroenterol.*, 15: 2960-2974.
- Muntané, J., F.J. Rodríguez, O. Segado, A. Quintero, J.M. Lozano, E. Siendones, C.A. Pedraza, M. Delgado, F. O'Valle, R. García, J.L. Montero, M. De La Mata and G. Miño, 2000. TNF-alpha dependent production of inducible nitric oxide is involved in PGE (1) protection against acute liver injury. *Gut*, 47: 553-562.
- Nagi, M.N. and H.A. Almakki, 2009. Thymoquinone supplementation induces quinone reductase and glutathione transferase in mice liver: possible role in protection against chemical carcinogenesis and toxicity. *Phytother Res.*, 23: 1295-1298.
- Nagi, M.N. and M.A. Mansour, 2000. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. *Pharmacol Res.*, 41: 283-289.
- Nagi, M.N., H.A. Almakki, M.M. Sayed-Ahmed and A.M. Al-Bekairi, 2010. Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. *Food Chem Toxicol.*, 48: 2361-2365.
- Nagi, M.N., K. Alam, O.A. Badary, O.A. Al-Shabanah, H.A. Al-Sawaf and A.M. Al-Bekairi, 1999. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem Mol Biol Int.*, 47: 153-159.
- Naik, S.R. and V.S. Panda, 2007. Antioxidant and hepatoprotective effects of *Ginkgo biloba* phytosomes in carbon tetrachloride-induced liver injury in rodents. *Liver Int.*, 27: 393-399.
- Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.*, 95: 351-358.
- Pushpavalli, G., C. Veeramani and K.V. Pugalendi, 2008. Influence of Piper betle on hepatic marker enzymes and tissue antioxidant status in D-galactosamine-induced hepatotoxic rats. *J Basic Clin Physiol Pharmacol.*, 19: 131-150.
- Quintero, A., C.A. Pedraza, E. Siendones, A.M. Kamal ElSaid, A. Colell, C. García-Ruiz, J.L. Montero, M. De la Mata, J.C. Fernández-Checa, G. Miño and J. Muntané, 2002. PGE1 protection against apoptosis induced by D-galactosamine is not related to the modulation of intracellular free radical production in primary culture of rat hepatocytes. *Free Radic Res.*, 36: 345-355.
- Raza, M., A.A. Alghasham, M.S. Alorainy and T.M. El-Hadiyah, 2006. Beneficial interaction of thymoquinone and sodium valproate in experimental models of epilepsy: reduction in hepatotoxicity of valproate. *Sci Pharm.*, 74: 159-173.

Seçkin, S., N. Koçak-Toker, M. Uysal and B. Oz, 1993. The role of lipid peroxidation and calcium in galactosamine induced toxicity in the rat liver. *Res Commun Chem Pathol Pharmacol.*, 80: 117-120.

Shakil, A.O., D. Kramer, G. Mazariegos, J.J. Fung and J. Rakela, 2000. Acute liver failure: clinical features, outcome analysis, and applicability of prognostic criteria. *Liver Transpl.*, 6: 163-169.

Vaca, C.E., J. Wilhelm and M. Harms-Rihdsahl, 1988. Interaction of lipid peroxidation product with DNA. A review. *Mutat Res -Reviews in Genetic Toxicology*, 195: 137-149.

Wang, X., B. Thomas, R. Sachdeva, L. Arterburn, L. Frye, P.G. Hatcher, D.G. Cornwell and J. Ma, 2006. Mechanism of arylating quinone toxicity involving Michael adduct formation and induction of endoplasmic reticulum stress. *Proc Natl Acad Sci USA*, 103: 3604-3609.

Wills, P.J. and V.V. Asha, 2006. Protective effect of *Lygodium flexuosum* (L.) Sw. (Lygodiaceae) against D-galactosamine induced liver injury in rats. *J Ethnopharmacol.*, 108: 116-123.

Ying-Wan, Y.L. Wu, X.C. Feng, L.H. Lian, Y.Z. Jiang and J.X. Nan, 2010. The protective effects of total saponins from *Ornithogalum saundersiae* (Liliaceae) on acute hepatic failure induced by lipopolysaccharide and D-galactosamine in mice. *J Ethnopharmacol.*, 132: 450-455.

Zhou, Y., C.M. Park, C.W. Cho and Y.S. Song, 2008. Protective effect of pinitol against D-galactosamine-induced hepatotoxicity in rats fed on a high-fat diet. *Biosci Biotechnol Biochem.*, 72: 1657-1666.