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The ameliorative effect of thymol against hydrocortisone-induced hepatic oxidative stress injury in adult male rats

Hanaa R. Aboelwafa and Hany N. Yousef

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Abstract: The aim of the present study was to investigate whether hydrocortisone induces oxidative stress in hepatocytes and to evaluate the possible ameliorative effect of thymol against such hepatic injury. Twenty-four adult male rats were divided into control, thymol, hydrocortisone, and hydrocortisone+thymol groups. The 4 groups were treated daily for 15 days. Hydrocortisone significantly induced oxidative stress in the liver tissues, marked by increased serum levels of alanine transaminase (ALT), aspartate transaminase (AST), total oxidative capacity (TOC), and tumor necrosis factor-alpha (TNF-α) accompanied by marked decline of serum levels of total protein, albumin, and total antioxidant capacity (TAC). Also, marked elevation in the levels of the thiobarbituric acid reactive substances (TBARS) and TNF-α, beside significant decrease in the level of glutathione (GSH) in hepatic tissues were recorded. These biochemical alterations were accompanied by histopathological changes marked by destruction of the normal hepatic architecture, in addition to ultrastructural alterations represented by degenerative features covering almost all the cytoplasmic organelles of the hepatocytes. Supplementation of hydrocortisone-treated rats with thymol reversed most of the biochemical, histological, and ultrastructural alterations. The results of our study confirm that thymol has strong ameliorative effect against hydrocortisone-induced oxidative stress injury in hepatic tissues.

Key words: antioxidants, glucocorticoids, liver, oxidative stress, thymol.

Résumé : Le but de l'étude présente était d'examiner si l'hydrocortisone induit un stress oxydant chez les hépatocytes et de déterminer si le thymol peut améliorer la situation, dans le cas d'un tel dommage hépatique. Vingt quatre rats mâles adultes ont été répartis en groupes contrôle, thymol, hydrocortisone et hydrocortisone + thymol. Les quatre groupes ont été traités quotidiennement pendant 15 jours. L'hydrocortisone induisait de manière significative un stress oxydant dans le tissu hépatique, caractérisé par des niveaux sériques accrus d'alanine transaminase (ALT), d'aspartate transaminase (AST), de la capacité oxydante totale (TOC) et du facteur nécrosant des tumeurs-alpha (TNF-α), accompagnés d'un déclin marqué des niveaux sériques de protéines totales, d'albumine et de la capacité antioxydante totale (TAC). De même, une élévation marquée des niveaux de substances réactives à l'acide thiobarbiturique (TBARS) et de TNF-α, en plus d'une diminution significative du niveau de glutathion (GSH) dans les tissus hépatiques ont été enregistrées. Ces modifications biochimiques étaient accompagnées de changements histopathologiques caractérisés par une destruction marquée de l'architecture hépatique normale, en plus de modifications ultrastructurales représentées par des caractéristiques dégénératives touchant presque tous les organites cytoplasmiques des hépatocytes. Une supplémentation des rats traités à l'hydrocortisone en thymol renversait la plupart des modifications biochimiques, histologiques et ultrastructurales. Les résultats des auteurs confirment que le thymol possède un fort potentiel d'amélioration du dommage oxydant induit par l'hydrocortisone dans les tissus hépatiques. [Traduit par la Rédaction]

Mots-clés : antioxydants, glucocorticoïdes, foie, stress oxydant, thymol.

Introduction

Hydrocortisone is a synthetic glucocorticoid (GC), employed as anti-inflammatory and immunosuppressant drug. It is widely administrated for the treatment of various types of diseases (Schimmer and Parker 2006; Wang et al. 2007; Bornstein 2009). In spite of the useful applications assigned to hydrocortisone, it has been associated with many serious adverse effects, especially when used at high doses and for prolonged periods (Schimmer and Parker 2006; Anagnostis et al. 2009; Coutinho and Chapman 2011).

Oxidative stress is a general term used to define the challenge of cells or tissues to high levels of reactive oxygen species (ROS). This can be due to overproduction of ROS in the cells or to the exposure of the cells to high external environmental levels of a variety of factors including tobacco smoke, pollutants, ionizing radiation, and synthetic drugs (Halliwell and Gutteridge 2007). ROS can cause oxidative damage to proteins, lipids, enzymes, and DNA, and they have also been linked to pathogenesis of oxidative diseases (Halliwell 1997). ROS and other free radicals are critical intermediates in the normal physiology and pathophysiology of hepatocytes (Diesen and Kuo 2010).

Although living cells possess an excellent scavenging mechanism to avoid excess ROS-induced cellular injury, these mechanisms become inefficient under the influence of external stresses, and as a result dietary supplementation of synthetic antioxidants is required. In recent years, due to toxicological concerns associated with the use of synthetic substances in food and increasing awareness about natural foods, there has been an increased interest in the use of natural substances as food preservatives and antioxidants (Peschel et al. 2006). In this context, natural products offer hope for the development of new medicines, most of which are plant-based. For instance, thymol is a dietary monoterpene phenol derivative of cymene, which is found in the oils of thyme and plants such as *Thymus vulgaris* and citrus plants (Archana et al. 2011). It is widely used in the pharmaceutical, cosmetic, and perfume

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industries, also for flavoring and preservation of several food products (Bauer et al. 1997). Thymol possesses multiple biological activities including anti-inflammatory (Tsai et al. 2011), hypocholesteraemic (Case et al. 1995), anti-lipid peroxidative (Alam et al. 1999), and free radical scavenging (Loziene et al. 2007) properties. Also, thymol exerts antioxidant activities in both cellular (Ünderger et al. 2009) and in vitro (Amiri 2012) models.

There are very few data in the literature about GCs excess and their relationship with free radical parameters. Therefore, the present study was undertaken to investigate whether hydrocortisone induces oxidative stress in hepatocytes, and whether such deleterious effects could be reversed by improvement of the antioxidant defense status of these cells using thymol as a natural antioxidant.

Materials and methods

Pharmacological materials

Hydrocortisone sodium succinate was available under the trade name Solu-Cortef in the form of 100 mg/2 mL bacteriostatic water for injection. It was obtained from Egyptian Int. Pharmaceutical Industries Co. (E.I.P.I.Co.). Thymol was purchased from Sigma-Aldrich Chemical Co. (St Louis, Missouri, USA). Corn oil was obtained from local market. All other chemicals used in the present study were of analytical grade of Merck quality.

Experimental animals

The present investigation was carried out on 24 adult male albino rats (*Rattus norvegicus*) of similar age (3–4 months) and weight (160–180 g). They were obtained from the animal house of Theodor Bilharz Research Institute (TBRI), El-Giza, Egypt and were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given pellet rodent diet, in addition to milk and water ad libitum. The rats were maintained under standard laboratory conditions with a 12 h light/dark cycle, temperature range of 25 ± 2 °C, and relative humidity of 55 ± 5% throughout the experimental period. All animal experiments were performed under protocols approved by the local Institutional Animal Ethics Committee of Ain Shams University.

Experimental design

The rats were divided randomly into 4 groups of 6 animals each. They were treated daily at 9 a.m. for 15 days as follows.

Group I (control group): Rats were intramuscularly (i.m.) injected with bacteriostatic water (0.1 mL/100 g bw) and orally received corn oil (1 mL/100 g bw) by gastric tube.

Group II (thymol group): Rats were orally given thymol (30 mg/100 g bw) dissolved in 1 mL corn oil by gastric tube. This level of administration is lower than the reported 98 mg/100 g bw as the median lethal dose for rats (Clark 1995), and it is the same to that used in previous research (Alam et al. 1999; Al-Malki 2010).

Group III (hydrocortisone group): Rats were i.m. injected with hydrocortisone sodium succinate (5 mg/100 g bw) dissolved in 0.1 mL bacteriostatic water. This dose was chosen according to the work accomplished by Artsruni et al. (2006) and Gevorgyan et al. (2008).

Group IV (hydrocortisone+thymol group): Rats were i.m. injected with hydrocortisone sodium succinate (5 mg/100 g bw) and were orally given thymol (30 mg/100 g bw).

Blood collection and tissue preparations

At the end of the experiment, all animals were fasted overnight and then anaesthetized using a slight diethyl ether exposure. Blood samples were collected by cardiac puncture procedure using sterile disposable syringes. Serum was separated by centrifugation at 1600*g* for 10 min and stored at −80 °C for further biochemical analyses. Rats were then sacrificed, and their livers were excised immediately. For biochemical estimations, samples of liver tissues of each animal was weighed and homogenized in phosphate buffer (pH 7.4) with a Teflon homogenizer at 4 °C. The homogenate was centrifuged at 291*g* at 4 °C for 10 min, and the supernatant was stored at −80 °C for various biochemical assays.

Biochemical estimations

Serum samples were analyzed to estimate the levels of aspartate transaminase (AST) and alanine transaminase (ALT) according to King (1965), total protein by the Biuret method (Armstrong and Carr 1964), albumin concentration (Doumas et al. 1971), as well as total antioxidant capacity (TAC) and total oxidative capacity (TOC) according to the method described by Koracevic et al. (2001) using ELISA commercial kit (Labor Diagnostika Nord GmbH & Co. KG, Germany). Concentrations of tumor necrosis factor-alpha (TNF-α) in sera and tissue homogenates were measured using rat TNF-α assay kit (Immuno-Biological Laboratories Co., Ltd., Japan) following the manufacturer's protocol. Liver reduced glutathione (GSH) was measured using a colorimetric method at 412 nm (BioAssay Systems QuantiChrom Glutathione Assay Kit, USA), while the level of liver thiobarbituric acid reactive substances (TBARS) was measured using a commercial assay kit (NWLSS, Washington, USA) according to the manufacturer's instructions.

Histological preparations

Small pieces of the liver from the control and experimental groups were removed and fixed rapidly in aqueous Bouin's fixative for 24 h. Then they were subjected to the normal procedures for paraffin sectioning. After routine processing, 4 μm sections were cut, stained with haematoxylin-eosin (H&E), dehydrated in ascending series of ethyl alcohol, cleared in xylene and mounted in DPX (Bancroft and Gamble 2002). The stained sections were examined with a light microscope and photomicrographs were made as required.

Ultrastructural preparations

Freshly excised liver samples were cut into small blocks, fixed directly in cold 4F1G (4% formalin + 1% glutaraldehyde adjusted at pH 2.2) for 24 h, then were post-fixed in 1% osmium tetroxide in 0.1 mol/L phosphate buffer (pH 7.3). After fixation, they were subjected to the normal procedures for ultrastructural evaluation by transmission electron microscopy as described previously by Dykstra et al. (2002). After routine processing, the stained grids were examined and photographed by JEM-1400-EX electron microscope (JEOL) at the Central Laboratory of Faculty of Science, Ain Shams University.

Statistical analysis

The results were expressed as mean ± SEM of 6 rats per group, and the statistical significance was evaluated by one-way analysis of variance (ANOVA) using the SPSS/17.0 software followed by LSD. Values were considered statistically significant when $P < 0.05$.

Results

Biochemical analysis

Table 1 depicts that administration of hydrocortisone sodium succinate for 15 days caused a significant elevation ($P < 0.05$) in the activities of AST (174.19%) and ALT (532.91%), paralleled with a significant decline ($P < 0.05$) in total protein (−20.13%) and albumin (−27.51%) when compared to the control group. Concomitant administration of thymol with hydrocortisone significantly lowered the disturbances in the measured parameters that occurred following treatment with hydrocortisone alone. Administration of thymol alone did not affect the values of these indices compared to the control group.

In the present investigation, various oxidative stress parameters (TAC, TOC, GSH, and TBARS) were evaluated in sera and (or) liver tissues of control and experimental groups. As illustrated in Table 2, hydrocortisone had induced significant decreases ($P < 0.05$) in serum TAC (−32.54%) and levels of GSH in liver tissues (−42.48%),

Table 1. Levels of transaminases (AST and ALT), total protein, and albumin in sera of rats from control and experimental groups.

	Groups			
Parameters	Control	Thymol	Hydrocortisone	Hydrocortisone+thymol
AST (U/L)	39.17±1.30[a]	38.12±1.02[a]	107.40±1.88[b]	68.56±1.06[c]
ALT (U/L)	21.33±0.60[a]	20.89±0.75[a]	135.00±3.20[b]	77.80±2.15[c]
Total protein (g/dl)	6.11±0.04[a]	6.02±0.07[a]	4.88±0.05[b]	5.23±0.08[c]
Albumin (g/dl)	4.18±0.07[a]	4.11±0.02[a]	3.03±0.09[b]	3.52±0.06[c]

Note: Values are expressed as mean ± SEM for 6 rats in each group. In each row, means with different superscript letters differ significantly at 5% ($P < 0.05$) level of significance. AST, aspartate transaminase; ALT, alanine transaminase.

Table 2. Levels of oxidative stress parameters in sera and liver tissues of rats from the control and experimental groups.

		Groups			
	Parameters	Control	Thymol	Hydrocortisone	Hydrocortisone+thymol
Serum	TAC (mmol/L)	1.69±0.017[a]	1.64±0.041[a]	1.14±0.03[b]	1.24±0.02[c]
	TOC (mmol/L)	0.29±0.014[a]	0.28±0.024[a]	0.65±0.02[b]	0.43±0.02[c]
Liver	GSH (μM/mg)	1.13±0.02[a]	1.11±0.06[a]	0.65±0.02[b]	0.88±0.03[c]
	TBARS (μM/mg)	12.99±0.65[a]	13.87±0.45[a]	50.23±0.83[b]	39.96±1.63[c]

Note: Values are expressed as mean ± SEM for 6 rats in each group. Within each row, means with different superscript letters differ significantly at 5% ($P < 0.05$) level of significance. TAC, total antioxidant capacity; TOC, total oxidative capacity; GSH, glutathione; TBARS, thiobarbituric acid reactive substances.

Table 3. Levels of the inflammatory cytokine (TNF-α) in sera and liver tissues of rats from control and experimental groups.

		Groups			
	Parameters	Control	Thymol	Hydrocortisone	Hydrocortisone+thymol
Serum	TNF-α (pg/mg)	2.86±0.02[a]	2.79±0.011[a]	5.26±0.179[b]	4.34±0.119[c]
Liver	TNF-α (pg/mg)	76.46±0.34[a]	78.33±0.14[a]	133.94±2.90[b]	105.26±2.40[c]

Note: Values are expressed as mean ± SEM for 6 rats in each group. Means with different superscript letters within the same row differ significantly at 5% ($P < 0.05$) level of significance. TNF-α, tumor necrosis factor-alpha.

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accompanied with significant increases ($P < 0.05$) in serum TOC (124.14%) and levels of TBARS in liver tissues (286.68%) post 15 days of treatment compared to the corresponding control group. Supplementation of thymol to the rats treated with hydrocortisone resulted in modulation of the measured oxidative stress parameters (but still significantly different when compared to control group). Also, Table 2 revealed non-significant changes in all the measured oxidative stress parameters in thymol-treated rats compared to the control group.

Table 3 shows that TNF-α levels were significantly ($P < 0.05$) increased in sera (83.92%) and hepatic tissues (75.18%) of rats treated with hydrocortisone relative to the corresponding control rats. On the other hand, oral administration of thymol with hydrocortisone improved significantly ($P < 0.05$) the TNF-α levels of both the sera and hepatic tissues when compared to the rats exposed to hydrocortisone alone. Rats treated only with thymol did not have significant changes in levels of TNF-α in sera or hepatic tissues when compared to control rats.

Histological observations

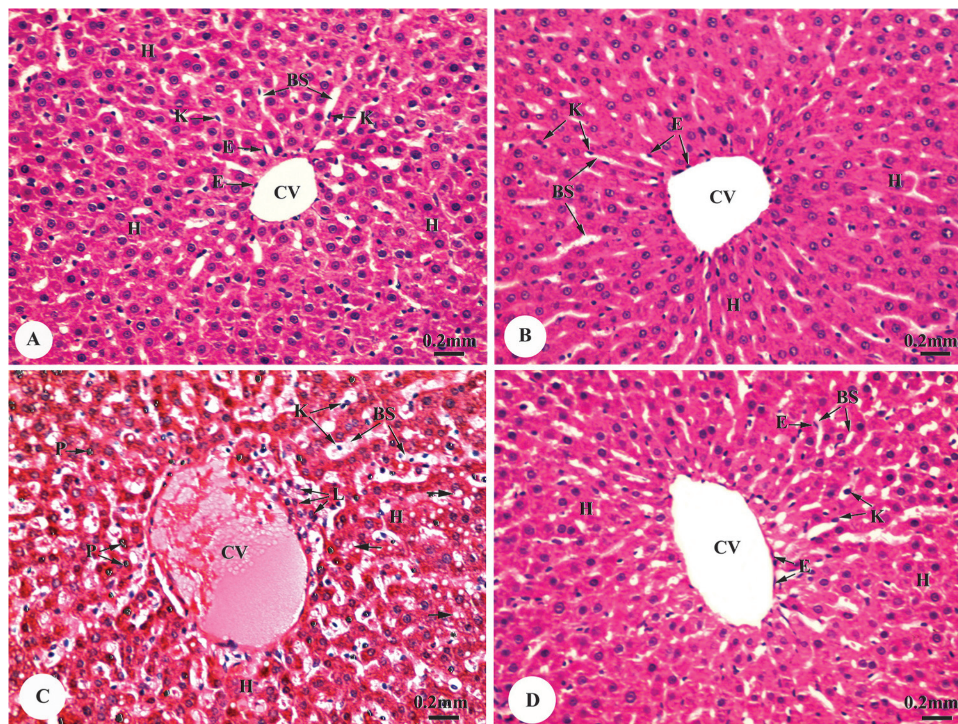
Light microscopic examination of the centrilobular zones of control (Fig. 1A) and thymol-treated (Fig. 1B) rats showed normal parenchymal architecture of the hepatic tissues. In contrast, rats receiving hydrocortisone showed massive pathological changes (Fig. 1C); the hepatic cords had lost their familiar organization, and the majority of hepatocytes showed fatty degeneration with pyknotic nuclei. The central veins displayed dilatation and congestion, with accumulation of lymphocytic inflammatory cells around their walls. The endothelial lining of most of these veins appeared eroded and demolished in some parts. In addition, the blood sinusoids were dilated and congested with swollen Kupffer cells detached from the borders of the sinusoids. On the other hand, the rats injected with hydrocortisone along with thymol displayed marked improvement in the hepatic architecture, and most of their hepatocytes revealed no signs of fatty degeneration (Fig. 1D).

Light microscopic examination of the periportal zones of hepatic lobules of control (Fig. 2A) and thymol-treated (Fig. 2B) rats showed normal portal tracts with normal hepatic architecture. Whereas in hydrocortisone-treated rats (Fig. 2C), the portal veins were highly widened with abnormal shapes and severely engorged with dense masses of stagnant blood. Also, the portal arteries and the bile ductules exhibited devastation. The hepatocytes obviously displayed disorganization and fatty degeneration. The liver sections from the rats treated with hydrocortisone concomitant with thymol showed marked improvement in the periportal zones and no signs of fatty degeneration were observed in the hepatocytes (Fig. 2D).

Ultrastructural observations

Transmission electron microscopic examination of the hepatocytes of control (Fig. 3A) and thymol-treated (Fig. 3B) rats showed normal fine structural organization. The cytoplasm embodied numerous scattered mitochondria, usually round to oval in shape, having short narrow transverse cristae and medium electron dense matrices; rough endoplasmic reticulum were observed as thin, parallel cisternae; also, smooth endoplasmic reticulum were observed as small agglomeration of tiny vesicles uniformly dispersed in the cytoplasm, which appeared to have a fine, granular appearance due to the presence of numerous glycogen rosettes and free ribosomes. Each hepatocyte possessed a spherical nucleus that had a nucleolus, peripheral heterochromatin, dispersed

Fig. 1. Light photomicrographs (A–D) of the centrilobular zones of hepatic tissues stained with H&E (400×) showing (A and B) normal radiating hepatic cords (H) extending around a central vein (CV) and separated by narrow blood sinusoids (BS), which are lined with endothelial (E) and Kupffer cells (K) in both control and thymol groups. (C) Disorganized hepatic cords (H) with hepatocytes having fatty degeneration (arrows) and pyknotic nuclei (P), dilated and congested blood sinusoids (BS) with swollen Kupffer cells (K), beside congested central vein (CV) with aggregation of lymphocytic inflammatory cells (L) around its eroded epithelium in hydrocortisone-treated rat. (D) Obvious improvement in the hepatic architecture in hydrocortisone-treated rat along with thymol.



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inner euchromatin, and was surrounded by a distinct nuclear envelope.

In contrast, the hepatocytes of rats treated with hydrocortisone displayed various hazardous ultrastructural changes of different severities (Figs. 3C and 3D). The rough endoplasmic reticulum cisternae and the smooth endoplasmic reticulum vesicles became hypertrophied and the mitochondria showed broken down cristae, weak electron dense matrices, and ruptured limiting membranes. The cytoplasm appeared vacuolated and possessed a large number of lysosomes, variable sized lipid droplets, as well as wide areas of glycogen rosettes. Conspicuous highly deformed nuclei, represented by destructive nuclear envelopes with prominent degree of chromatolysis, were present in most of the hepatocytes.

On the other hand, the hepatocytes of rats treated with hydrocortisone along with thymol (Figs. 3E and 3F) entirely confirmed the results of the histological examination. The hepatocytes showed nearly the typical fine structure of the control rat hepatocytes.

Discussion

The liver plays a vital role in the detoxification of endogenous waste products and exogenous chemicals (Moran Campbell et al. 1995). Accordingly, hepatocytes are particularly vulnerable to toxicity produced by reactive metabolites, since they are the major sites of xenobiotic metabolism. Many compounds, including clinically useful drugs, can cause cellular damage in the liver through metabolic activation of the chemicals to highly reactive compounds such as free radicals causing oxidative stress (Kumar et al. 1997).

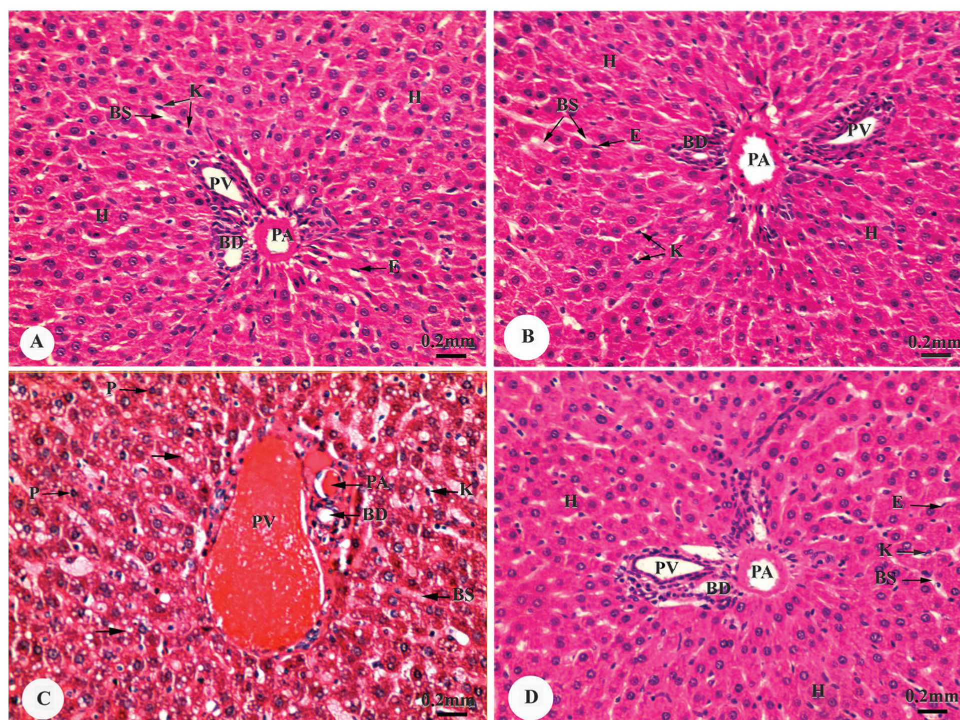
Chronically elevated levels of GCs have been intrinsically tied to fatty liver development (Andrews and Walker 1999). GCs are known to contribute to fatty liver production through a combination of increased fatty acid synthesis and decreased fatty acid β oxidation (Letteron et al. 1997), but their metabolic and molecular mechanisms in fatty liver development still remain largely elusive (Vegiopoulos and Herzig 2007).

The present study showed that hydrocortisone caused elevation in serum levels of AST and ALT, reflecting structural damage of liver cells, since these enzymes are cytoplasmic in location and released into the blood as a result of cellular damage (Wang et al. 1997; Chaung et al. 2003). Also, hydrocortisone caused significant decline in levels of serum total protein and albumin, suggesting a decrease of protein synthesis and (or) increase of protein catabolism (Abdel-Wahhab and Aly 2005).

Treatment of the hydrocortisone group with thymol brought back the levels of AST, ALT, albumin, and total protein near to the control levels, indicating maintenance of functional integrity of the hepatocytes. These results confirm those reported by El-Nekeety et al. (2011) and Hamzawy et al. (2012) in their studies on the antioxidant properties of *Thymus vulgaris* oil against aflatoxin (AFs)-induced oxidative stress in male rats.

Oxidative stress results in toxicity when the generated free radicals exceed the cell's capacity for their removal (Sies 1997). Measurement of TBARS, which are considered to be one of the main manifestations of oxidative damage and play an important role in toxicity and carcinogenicity, is one of the most frequently used markers for determining lipid peroxidation (LP) (Holley and Cheeseman 1993). In addition, estimation of TAC, which includes enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, as well as macromolecules such as albumin, ceruloplasmin, and ferritin, provide more relevant biological information compared with that obtained from measurement of individual components, because TAC considers the cumulative effect of all antioxidants present in plasma and body fluids (Gad et al. 2011). GSH is the main intracellular antioxidant in most mammalian

Fig. 2. Light photomicrographs (A–D) of the periportal zones of hepatic tissues stained with H&E (400×) showing (A and B) well-organized hepatic portal vein (PV), hepatic portal artery (PA), and bile ductule (BD), normal radiating hepatic cords (H) separated by narrow blood sinusoids (BS) lined with endothelial (E) and Kupffer cells (K) in control and thymol groups. (C) Dilatation and congestion of both the hepatic portal vein (PV) and hepatic portal artery (PA), dilated and congested blood sinusoids (BS) with swollen Kupffer cells (K), in addition to deformed hepatocytes with fatty degeneration (arrows), and pyknotic nuclei (P) in hydrocortisone-treated rat. (D) Normal structure of the portal tract with obvious improvement in the hepatic architecture in the rat treated with hydrocortisone in concomitant with thymol.



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cells, and it participates in the removal of hydrogen peroxide and toxic end products of LP (Rizzardini et al. 2003).

In the present study, the elevated levels of both TOC in sera and TBARS in liver tissues that accompanied significant declines in levels of TAC in sera and GSH in liver tissues of rats administrated with hydrocortisone suggest enhancement of lipid peroxidation, leading to excessive formation of free radicals. The produced free radicals initiate chain reactions of direct and indirect bond formation with cellular molecules, causing impairment of crucial cellular processes that may ultimately culminate in extensive cell damage and death (Halliwell 1994).

GCs have been shown to induce oxidative stress and to release mitochondrial cytochrome c (Hegardt et al. 2003; Tonomura et al. 2003). Also, Roma et al. (2009) reported that dexamethasone increased oxidative stress in insulin-producing cells, ultimately leading to apoptotic cell death, and pointed to an important role for hydrogen peroxide in the death of insulin-producing cells exposed to GCs.

Treatment of hydrocortisone-intoxicated rats with thymol significantly reversed the changes in all the estimated oxidative stress parameters, indicating that thymol has an ameliorative effect against hydrocortisone-mediated liver injury through its free radical scavenging and antioxidant properties (Loziene et al. 2007; Ünderger et al. 2009). The current result supports the report of Alam et al. (1999) that thymol decreased ROS production, resulting in inhibition of hepatotoxicity induced by carbon tetrachloride (CCl_4) in male Swiss albino mice.

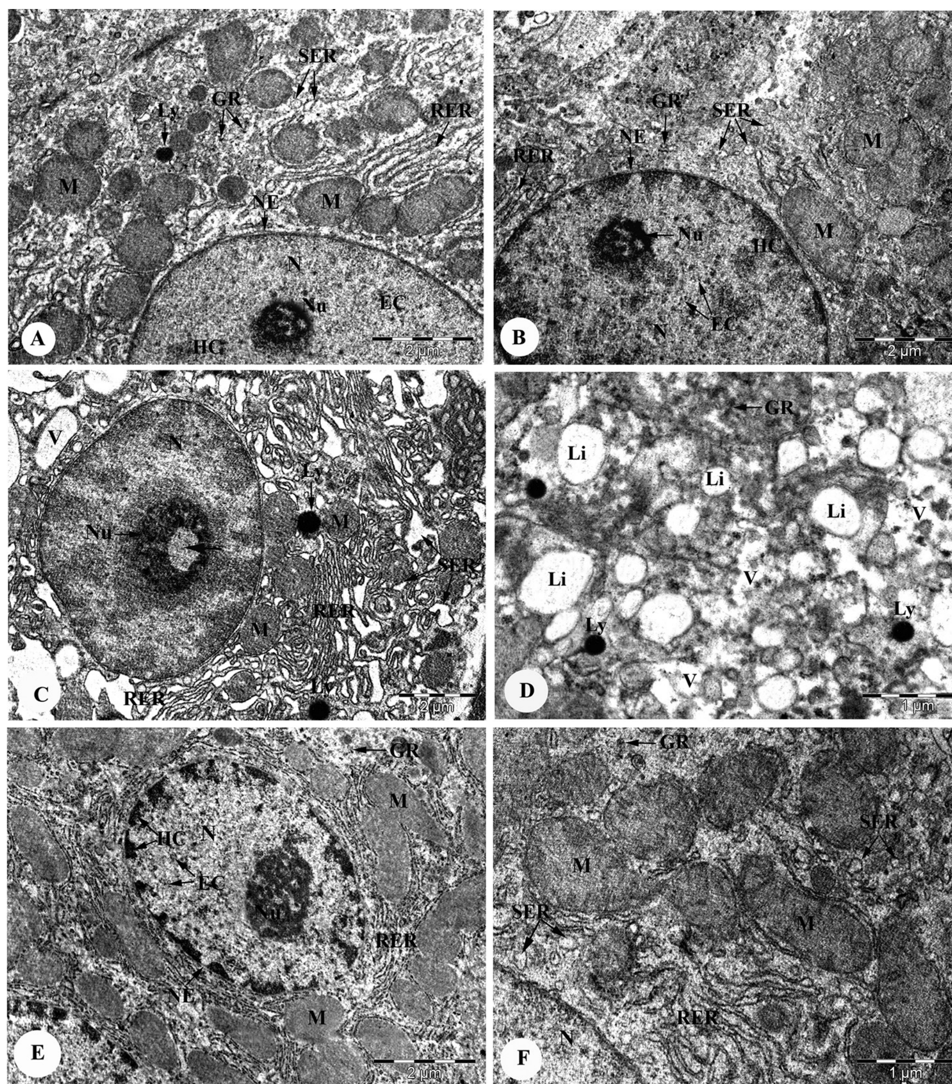
TNF-α is produced by macrophages and plays an important role in tumor promotion (Suganuma et al. 2000). It exhibits a causal role in the development of liver injury (Barton et al. 2001). In the present investigation, treatment of rats with hydrocortisone significantly increased TNF-α in sera and hepatic tissues, suggesting that hydrocortisone preferentially affects macrophage functions and induces the development of liver injury. On the other hand, oral administration of thymol concomitant with hydrocortisone treatment significantly reduced TNF-α level in both sera and hepatic tissues. These results confirm those reported by Hamzawy et al. (2012). Similarly, Liang et al. (2014) showed that thymol markedly inhibited the production of TNF-α in lipopolysaccharide-stimulated mouse mammary epithelial cells. The mechanism by which thymol possesses an anti-inflammatory response may be due to its inhibitory effect on the expression of TNF-α gene (Karimian et al. 2013).

The biochemical results have been confirmed by the histological and ultrastructural observations. Among the prominent lesions encountered following hydrocortisone treatment were the swelling and prominence of Kupffer cells. This lesion is attributed to the important role of Kupffer cells in the defense mechanisms of the body, since they are first exposed to the harmful substances following into the liver through the portal vein and contribute to the removal of tumor cells migrating to the liver (Wang et al. 1995; Enomoto et al. 2000).

The apparent fatty degeneration and cellular necrosis in the hepatic tissues of rats post hydrocortisone treatment confirmed the elevated levels of AST and ALT in sera. The leakage of large quantities of serum enzymes into the blood stream was reported to be associated with massive centrilobular necrosis, fatty degeneration, and cellular infiltration of liver (Huang et al. 2012). In the same context, similar findings were recorded by Safaei et al. (2011) following dexamethasone treatment.

At the ultrastructural level, the endoplasmic reticulum showed marked proliferation and hypertrophy. Such damage can be explained in view of Cribb et al. (2005), who reported that the endoplasmic reticulum is an important target for damage by reactive

Fig. 3. Electron photomicrographs (A–F) of hepatocytes of control and experimental groups showing (A and B) intact nuclei (N) enclosed within distinct nuclear envelopes (NE) and possessing electron dense nucleoli (Nu), heterochromatin (HC), and fine euchromatin (EC) in control and thymol groups. Besides, numerous mitochondria (M), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), lysosomes (Ly), and glycogen rosettes (GR) are seen in the cytoplasm. (9000×). (C and D) severe deteriorated cytoplasm with marked dilatation and proliferation of both the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER), deformed mitochondria (M), numerous lysosomes (Ly), vacuoles (V), variable sized lipid droplets (Li), and accumulation of glycogen rosettes (GR), as well as deformed nucleus (N) displaying prominent degree of chromatolysis and vacuolation of its nucleolus (arrow) in hydrocortisone-treated rats. (9000× and 14 000×). (E and F) obvious improvement in the fine structure of most of the cytoplasmic organelles and the nuclei of hydrocortisone-treated rats along with thymol (9000× and 14 000×).



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intermediates. This is due to the initial involvement of the endoplasmic reticulum through its host by xenobiotics-metabolizing enzymes that are involved in the bioactivation and detoxification of numerous xenobiotics. Also, this endoplasmic reticulum damage explains the decrease in levels of total serum proteins encountered in the biochemical results, reflecting impaired protein synthesis capacity of the hepatocytes.

In the present study, disruption of the normal mitochondrial structure post hydrocortisone treatment may be attributed to the overproduction of ROS (Halliwell et al. 1995). Also, the increased number of lysosomes is an indicator of focal cytoplasmic degradation, a process in which lysosomes are associated with the digestion of damaged portions of the hepatocyte cytoplasm (Phillips et al. 1987).

The clear accumulation of lipid droplets in the cytoplasm of hepatocytes following hydrocortisone administration may be attributed to inhibition of the mitochondrial fatty acid β-oxidation (Letteron et al. 1997). In the same context, Begriche et al. (2011) mentioned that liver triglycerides accumulate as large lipid vacuoles, displacing the nuclei at the periphery of the hepatocytes post treatment with GCs. Also, glycogen accumulation may be observed in hepatocytes as a manifestation of toxicity. This is apparently due to impairment of enzymatic activity for glycogen catabolism or an increase in glycogen synthesis (Haschek and Rousseaux 1991).

The severe destructive nuclear changes of hepatocytes after hydrocortisone application can be interpreted by the strong binding of this synthetic drug with DNA causing changes in gene expression, thereby affecting many cellular functions (Schimmer and Parker 2006; Clark et al. 2008).

On the other hand, co-adminstration of thymol with hydrocortisone showed obvious improvement in the structure and fine structure

of the hepatic tissues. The hepatoprotective activity of thymol can be explained due to stabilizing the hepatocyte membranes by preventing LP, ameliorating the activities of the antioxidant enzymes, as well as its free radical scavenging activity.

In conclusion, the present study provides new insights for the strong ameliorative effect of thymol against hydrocortisone-induced oxidative stress injury in hepatic tissues.

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