

## Original Article



## Antioxidant Machinery Related to Decreased MDA Generation by *Thymus Algeriensis* Essential Oil-induced Liver and Kidney Regeneration

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### Abstract

**Objective** This study was conducted to determine the histopathological and biochemical effects of *Thymus algeriensis* essential oil (TEO) on hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced oxidative stress in liver and kidney tissues of rats.

**Methods** Rats were treated in six groups and were exposed for 2 weeks to low (LD; 100 μmol/L) and high doses (HD; 1 mmol/L) of H<sub>2</sub>O<sub>2</sub> in the presence or absence of TEO (180 mg/kg). Liver and kidney atrophy was measured by using biochemical and histopathological assays.

**Results** Our study demonstrated that H<sub>2</sub>O<sub>2</sub> induced liver and kidney atrophy, as evidenced by the significant elevation of serum aminotransferase, urea, and creatinine levels compared with those in the control rats. Urea levels were estimated by evaluating the activity of serum urease that hydrolyzes urea into CO<sub>2</sub> and ammonia. However, TEO treatment significantly alleviated oxidative stress in the H<sub>2</sub>O<sub>2</sub>-induced liver and kidney toxicity model by reducing the levels of malondialdehyde concomitantly with marked elevations in superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase, as well as decrease in glutathione activity.

**Conclusion** Our data demonstrated that TEO protected against H<sub>2</sub>O<sub>2</sub> toxicity by decreasing oxidant levels and DNA damage, as well as increasing antioxidant levels, indicating that TEO has a spectrum of antioxidant and DNA-protective properties.

**Key words:** Liver and kidney atrophy; Hydrogen peroxide; *Thymus algeriensis*; Antioxidants; Toxicity

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### INTRODUCTION

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is an important cause of oxidative injury because it can easily transform into a hydroxyl radical, which is one of the most destructive free radicals. Moreover, the half-life of H<sub>2</sub>O<sub>2</sub> is comparatively longer than that of other reactive oxygen species (ROS)<sup>[1]</sup>. H<sub>2</sub>O<sub>2</sub> uses aquaporins to cross cell

membranes rapidly<sup>[2]</sup> and therefore has the ability to diffuse in and out through the cell membrane. In a previous study, it was found that H<sub>2</sub>O<sub>2</sub>, rather than the superoxide anion, is the most toxic species that induces alterations in cellular functioning<sup>[3]</sup>.

The liver is very susceptible to toxicity produced by reactive metabolites because it is a major site of xenobiotic metabolism. Other than the liver, the kidney, which is susceptible to toxicity, is

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also a highly specialized organ that maintains the internal environment of the body through selective excretion of unwanted substances or retention of various substances according to the needs of the body<sup>[4]</sup>. ROS have been implicated in the pathogenesis of several harmful diseases, including multiple sclerosis and liver cirrhosis. It is also a universal risk factor for the development of liver cancer (human hepatocellular carcinoma)<sup>[5]</sup>. Because hepatocyte inflammation is the major cause of liver disorders, one important therapeutic strategy may include the use of anti-inflammatory and hepatoprotective interventions, either alone or in combination<sup>[6]</sup>. Furthermore, the use of safe natural compounds as anti-inflammatory agents could be a better alternative for the detoxification of the liver and kidney.

Numerous studies revealed that plant remedies are useful in treating liver diseases<sup>[7]</sup>. As many plants and herbs have effective natural antioxidants, such as carboic acid, isoprene derivatives, carotene, terpene, and polyphenol,<sup>[8]</sup> extracts or essential oils from these plants could serve as effective hepatoprotective and nephroprotective agents. Collective evidence supports that H<sub>2</sub>O<sub>2</sub>-induced cell injury can be prevented by antioxidants such as the essential oil of *Thymus algeriensis* (TEO)<sup>[9]</sup>. As described in our previous reports, TEO has the potential to prevent acute gastric injury and testis toxicity in rats by correcting the cellular imbalance between oxidants and antioxidants<sup>[10-11]</sup>.

In this study, we determined whether TEO has the potential to protect the liver and kidney from H<sub>2</sub>O<sub>2</sub>-induced injuries. We present extensive data showing the biochemical and pathological alterations induced by H<sub>2</sub>O<sub>2</sub>, and the preventive effects of TEO against H<sub>2</sub>O<sub>2</sub>-induced tissue damage in rats.

## MATERIALS AND METHODS

### Chemicals

All reagents, including Ellman's reagent, reduced glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid), bovine serum albumin, H<sub>2</sub>O<sub>2</sub>, thiobarbituric acid (TBA), 2,4-dinitrochlorobenzene (CDNB), and Tris-HCl buffer were purchased from Sigma (St. Louis, MO, USA), Fluka Chemie (Buchs, Switzerland), and Merck (Nottingham, UK).

### Plant Material

The aerial portions of wild *Thymus algeriensis*

plants were collected during the flowering stage in March 2013 from Jebel Orbata in Gafsa, which is located on the middle west of Tunisia at a latitude and longitude of 34.39641 and 9.12914, respectively. Plant specimens (Voucher #1188) were identified and deposited in the herbarium of medicinal plants at the Agronomic National Institute of Tunis, Tunisia. The essential oil of TEO was extracted with a Clevenger apparatus<sup>[12]</sup> and dried over Na<sub>2</sub>SO<sub>4</sub>. Purified TEO was stored in sealed dark vials at 4 °C.

### Experimental Animals

Thirty-six male Sprague Dawley rats (6-8 weeks old) weighing between 180 and 200 g were obtained from the animal laboratory of the Pasteur Institute of Tunis. The Ethical Committee for animal experiments of the Faculty of Sciences of Bizerte, University of Carthage, Tunisia, approved all animal protocols (Ethic# LNSP/Pro 152012) governing the experiments. Before the experiment, rats were acclimatized for 7 days to human contact to minimize their physiological responses to handling for subsequent protocols<sup>[12]</sup>. Rats were randomized into six groups comprising six animals each for treatments as follows: (1) control (C), (2) low dose (100 µmol/L) H<sub>2</sub>O<sub>2</sub> (LD H<sub>2</sub>O<sub>2</sub>), (3) high dose (1 mmol/L) H<sub>2</sub>O<sub>2</sub> (HD H<sub>2</sub>O<sub>2</sub>), (4) TEO (180 mg/kg per day dissolved in normal saline), (5) TEO and LD H<sub>2</sub>O<sub>2</sub> (180 mg/kg per day and 100 µmol/L, respectively), and (6) TEO and HD H<sub>2</sub>O<sub>2</sub> (180 mg/kg per day and 1 mmol/L, respectively). TEO was administered 1 h prior to H<sub>2</sub>O<sub>2</sub> treatment in animals receiving both agents. Rats were then housed in a pathogen-free environment (clean polypropylene cages) with a 12 h light/dark cycle, relative humidity of 55%±10%, and a temperature of 20-25 °C. The animals were permitted access to standard pellets and water *ad libitum*. The dosage of TEO used had been optimized in our previous study<sup>[10]</sup>. All treatments were administered orally for 15 d.

Body weights were measured daily for 15 d. At the end of this experiment, rats were anesthetized and blood was collected from tail vein to measure the enzymatic activities. Subsequently, rats were euthanized by using cervical dislocation. The livers and kidneys of treated and untreated animals were carefully dissected, and portions were fixed in 10% buffered formaldehyde for routine histological evaluations after staining with hematoxylin and eosin (H&E). Unfixed portions of the livers and kidneys were weighed and subjected to extraction by using a mechanical rotary homogenizer in

0.1 mol/L phosphate buffer at a pH of 7.4. The supernatant obtained after centrifugation at  $8000 \times g$  for 15 min was used as a source of enzymes for biochemical analyses.

### Assessment of Hepatic and Renal Functional Marker Enzymes

The collected blood was also used for the estimation of serum marker enzymes. Serum urease activity was determined in the presence of urea by assessing  $\text{CO}_2$  and ammonia production. The addition of phenol-hypochloride led to formation of the indophenol-blue complex with an absorbance at  $600 \text{ nm}^{[13]}$ . Serum creatinine levels were estimated by using a reaction with picric acid in alkaline buffer to form a yellow-orange complex, whose color intensity is proportional to the creatinine concentration in the sample<sup>[14]</sup>. Alanine aminotransferase and aspartate aminotransferase activities were determined by using pyruvate and oxaloacetate as substrates, wherein NADH is converted into  $\text{NAD}^+$  proportional to the activities of those enzymes according to the method described by Wilkinson<sup>[15]</sup>.

### Assessment of Enzymatic Antioxidants and Lipid Profile

The supernatant was collected for the estimation of the protein concentration by using the Lowry method<sup>[16]</sup>, using crystalline bovine serum albumin as a standard. The level of malondialdehyde (MDA) in liver tissue homogenates was estimated by measuring TBA-reactive substances by using the TBA method and expressed as nmoles of MDA/mg protein<sup>[17]</sup>.

Antioxidant levels were evaluated by measuring the activities of superoxide dismutase (SOD)<sup>[18]</sup>, catalase (CAT)<sup>[19]</sup>, glutathione peroxidase (GPx)<sup>[20]</sup>, and glutathione transferase (GST)<sup>[21]</sup>, and the levels of reduced glutathione (GSH)<sup>[22]</sup>. The specific activities of the antioxidant enzymes (SOD, CAT, GST, and GPx) and antioxidant levels (GSH) were expressed in U/mg protein.

### Histopathological Examination

For histopathological observations, a portion of the liver and kidney from each animal was preserved in 10% neutral buffered formalin and processed for paraffin embedding by using the standard methods described by Drury and Wallington<sup>[23]</sup>. Tissue sections were prepared and stained with H&E.

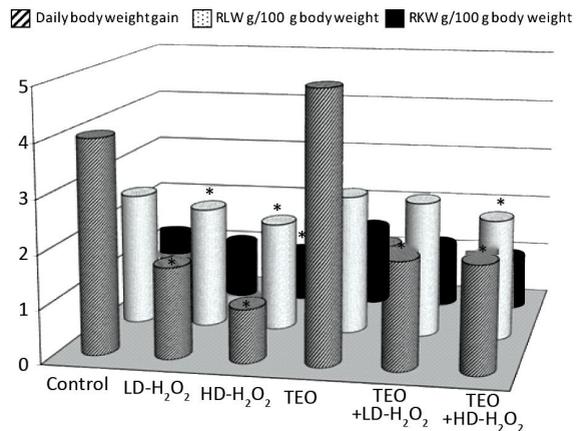
### Statistical Analysis

Statistical analysis was performed by using Statistica software version 5.1 and Microsoft Excel software. The significance of the differences between various groups was analyzed by using one-way analysis of variance followed by Duncan's multiple range tests. The level of significance was set at  $P < 0.05$ .

## RESULTS

### Body, Liver, and Kidney Weights

In all experimental groups, rats remained active and vigorous throughout the treatment duration. The body and relative organ weights of untreated, low- and high-dose  $\text{H}_2\text{O}_2$ -exposed, and TEO-pretreated animals were examined. The results showed that treatment with both high and low doses of  $\text{H}_2\text{O}_2$  induced a significant ( $P < 0.05$ ) body weight loss in rats compared to that in controls after 2 weeks (Figure 1). At dosages of both  $100 \mu\text{mol/L}$  and  $1 \text{ mmol/L}$ ,  $\text{H}_2\text{O}_2$  induced dyspnea and paralysis. However, body and organ weights were found to be enhanced on TEO administration. Significant reductions in relative liver and kidney weights were also observed in  $\text{H}_2\text{O}_2$ -treated animals. However, administration of TEO prevented  $\text{H}_2\text{O}_2$ -induced liver, kidney, and weight loss (Figure 1).



**Figure 1.** Change in body weight (BW), relative liver weight [RLW (g/100 g BW)] and relative kidney weight [RKW (g/100 g BW)] between the start and end of the study (15 days) in control rats and rats receiving  $\text{H}_2\text{O}_2$  ( $100 \mu\text{mol/L}$  and  $1 \text{ mmol/L}$ ), TEO ( $180 \text{ mg/kg}$  per day), or a combination of the two. Values are expressed as means  $\pm$  SEM. \*  $P < 0.05$  vs. control.

### Effect of H<sub>2</sub>O<sub>2</sub> on Serum Hepatic and Kidney Markers

The effects of H<sub>2</sub>O<sub>2</sub> treatment as well as the preventive effects of TEO on serum biochemical markers are shown in Table 1. A significant increase ( $P<0.05$ ) in functional marker enzyme levels (AST and ALT) was observed in LD-H<sub>2</sub>O<sub>2</sub>- and HD-H<sub>2</sub>O<sub>2</sub>-treated rats compared to that in the untreated rats. However, prophylactic treatment with TEO before H<sub>2</sub>O<sub>2</sub> administration significantly attenuated these effects.

The levels of renal products, urea and creatinine, significantly increased in LD-H<sub>2</sub>O<sub>2</sub>- and HD-H<sub>2</sub>O<sub>2</sub>-treated rats compared to those in the control group. TEO significantly ( $P<0.05$ ) reduced renal serum product levels in H<sub>2</sub>O<sub>2</sub>-treated animals compared to that in the control group (Table 1). This indicates the possible nephroprotective efficacy of the thyme species against H<sub>2</sub>O<sub>2</sub> toxicity.

### Hepatic Lipid Profile and Antioxidant Enzyme Activities

Lipid peroxidation (oxidative degradation of lipids) is a widely accepted measure for assessing oxidative stress. The effects of H<sub>2</sub>O<sub>2</sub> treatment and the preventive effects of TEO on lipid peroxidation and antioxidant activity in liver tissue are shown in Figure 2. H<sub>2</sub>O<sub>2</sub>-mediated oxidative stress and antioxidant ability in rats was partially or completely inhibited by pretreatment with TEO (Figure 2A-G). The administration of H<sub>2</sub>O<sub>2</sub> led to a dose-dependent increase in MDA activity relative to that in control (Figure 2B). In addition, H<sub>2</sub>O<sub>2</sub> treatment at both 100 µmol/L and 1 mmol/L doses significantly reduced the activities of GST, GPx, GSH, CAT, and SOD (Figure 2C-G) in a dose-dependent manner compared to those in the control group. While pretreatment with TEO partially recovered CAT activity (Figure 2F) and

GSH levels (Figure 2E), it fully recovered SOD (Figure 2G) and GPx (Figure 2D) activities to those of the control group ( $P<0.05$ ). TEO induced a significant increase in GPx and GSH activities (Figure 2D, 2E) in liver tissues.

### Renal Lipid Profile and Antioxidant Enzyme Activities

The effects of H<sub>2</sub>O<sub>2</sub> treatment and the preventive effects of TEO on lipid peroxidation and antioxidant activity in the kidney are shown in Figure 3. H<sub>2</sub>O<sub>2</sub>-mediated oxidative stress and antioxidant ability in kidney tissues were partially or completely inhibited by TEO pretreatment (Figure 3A-G). Administration of H<sub>2</sub>O<sub>2</sub> increased MDA activity in a dose-dependent manner relative to that in the control (Figure 3B). However, MDA induction in the kidney was not as high as that in the liver. H<sub>2</sub>O<sub>2</sub> treatment significantly ( $P<0.05$ ) reduced the activities of GST, GPx, GSH, CAT, and SOD (Figure 3C-G) in a dose-dependent manner compared with those in the control group. Similar to the trend found in liver tissues, TEO pretreatment partially recovered CAT activity (Figure 3F) and GSH levels (Figure 3E), while fully recovering SOD (Figure 3G) and GPx (Figure 3D) activities to those of the control group ( $P<0.05$ ). TEO significantly induced the activity of GPx and levels of GSH (Figure 3D, 3G) in kidney tissues.

### Correlation among Total Protein, Antioxidant Enzymes, and Daily Body Weight Gain

Linear correlation coefficients were established to explore the trend of association among total protein, antioxidant enzymes, daily body weight gain, and hepatic MDA levels (Table 2). As presented in Table 2, AST ( $r=0.95$ ) and ALT ( $r=0.86$ ) levels showed a significant ( $P<0.05$ ) positive correlation with MDA levels. A similar result was obtained between

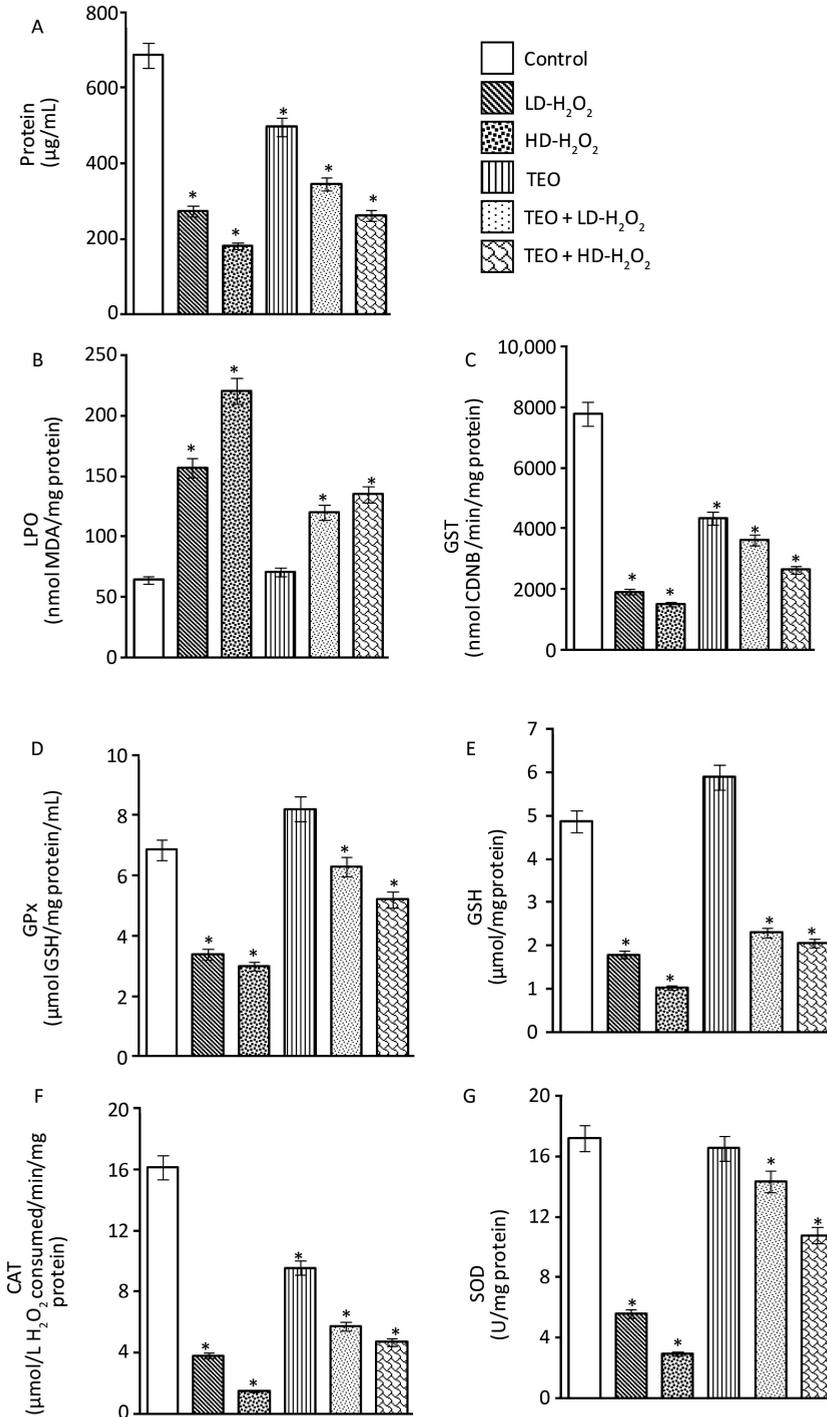
**Table 1.** Effect of TEO on Biochemical Parameters

Groups	AST (U/L)	ALT (U/L)	Urea (mmol/L)	Creatinine (µmol/L)
Control	84.60±2.12	66.50±4.23	5.33±0.00	37.66±0.10
LD H <sub>2</sub> O <sub>2</sub>	103.50±1.34*	76.00±0.48*	6.13±0.37	42.66±3.28
HD H <sub>2</sub> O <sub>2</sub>	106.60±3.18*	97.50±1.06*	6.36±2.56	45.00±2.42*
TEO	86.00±0.50	69.00±0.00	5.86±0.00	36.00±1.08
TEO+LD H <sub>2</sub> O <sub>2</sub>	96.33±1.02*	65.50±3.23	5.60±0.50	39.06±3.53
TEO+HD H <sub>2</sub> O <sub>2</sub>	100.30±1.68*	84.50±1.10*	4.66±0.42	41.66±0.38

**Note.** All values were expressed as mean±SEM. AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, GGT: gamma-glutamyl transferase. \*Significant value was at  $P<0.05$ .

urea ( $r=0.3$ ), creatinine ( $r=0.34$ ), and MDA. These results indicated the parallel evolution of the above-mentioned parameters with MDA levels. Therefore, to evaluate the contribution of total protein, antioxidant enzymes, and daily body weight

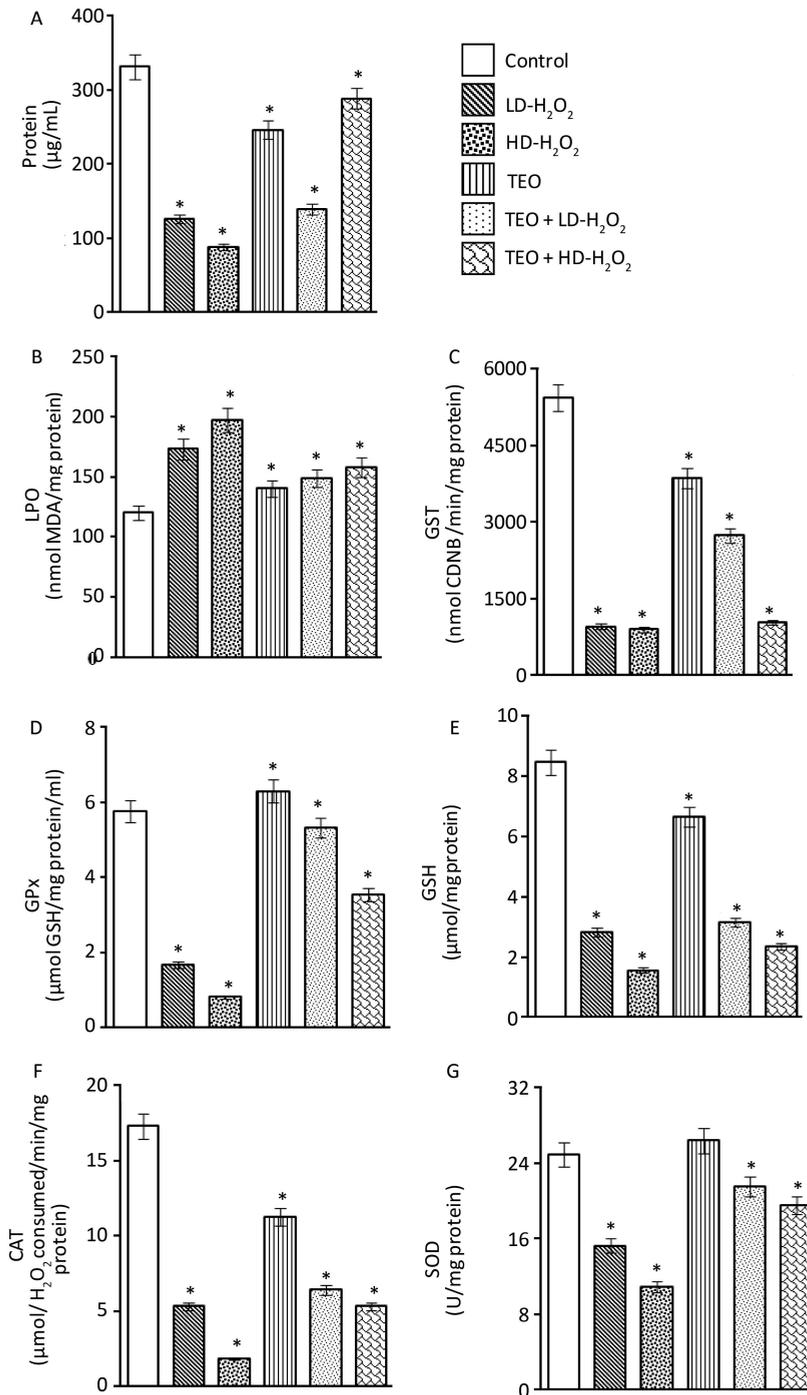
gain to renal MDA levels (Table 3) and its antioxidant capacity, linear correlation coefficients were determined. A negative ( $P<0.05$ ) correlation was found between protein concentration ( $r=-0.89$ ;  $r=-0.24$ ), CAT ( $r=-0.88$ ;  $r=-0.23$ ), SOD ( $r=-0.95$ ;  $r=-0.24$ ),



**Figure 2.** Variations of protein levels (A), lipid peroxidation (B), and GST (C), GPx (D), GSH (E), CAT (F), and SOD (G) activities in the livers of experimental rats after injection. Data are mean $\pm$ SEM,  $n=6$ . \*Significant Value was at  $P<0.05$ .

GPx ( $r=-0.94$ ;  $r=-0.24$ ), GST ( $r=-0.89$ ;  $r=-0.16$ ), GSH ( $r=-0.47$ ;  $r=-0.47$ ), and MDA in kidney and liver tissues, suggesting that antioxidant enzymes might be responsible, among other factors, for antioxidant

defense. There was no significant correlation between daily weight gain and MDA content in the liver, but a positive correlation ( $r=0.056$ ) was found in the kidney.



**Figure 3.** Variations protein levels (A), of lipid peroxidation (B), and GST (C), GPx (D), GSH (E), CAT (F), and SOD (G) activities in the kidneys of experimental rats after injection. Data are mean±SEM,  $n=6$ . \*Significant Value was at  $P<0.05$ .

### Histological Changes in the Liver and Kidney

Histological appearance plays an important role in the study of the hepatoprotective and renoprotective potential of TEO in rats. Representative photomicrographs of the control, H<sub>2</sub>O<sub>2</sub>-treated, and TEO-treated liver and kidney tissues are presented in Figures 4 and 5, respectively. Sections were stained with H&E to examine cell structure. The necropsy of the euthanized rats showed hemorrhage in the liver. Histological features of the livers of untreated rats appeared normal (Figure 4A). In contrast, in the H<sub>2</sub>O<sub>2</sub>-treated groups, higher doses of H<sub>2</sub>O<sub>2</sub> administered frequently (<3 weeks) caused hepatotoxicity to a larger extent

**Table 2.** Linear Correlation Coefficients (*r*) and *P* Value of Total Protein, Antioxidant Status, and Daily Body Weight Gain versus the Hepatic MDA Level

Antioxidant Parameters	LPO (nmol MDA/ mg protein)
	<i>R</i>
Proteins concentration (µg/mL)	-0.89
CAT (µmol H <sub>2</sub> O <sub>2</sub> consumed/min/mg protein)	-0.88*
SOD (U/mg of protein)	-0.95*
GPx (µmol GSH/mg protein/mL)	-0.94*
GST (nmol of CDNB conjugate Formed min <sup>-1</sup> mg <sup>-1</sup> protein)	-0.89*
GSH (µmol/mg protein)	-0.84*
AST (U/L)	0.95*
ALT (U/L)	0.86*
Daily body weight gain (gm)	-0.92

**Note.** \* Significant value was at *P*<0.05.

**Table 3.** Linear Correlation Coefficients (*r*) and *P* Value of Total Protein, Antioxidant Status, and Daily Body Weight Gain versus the Kidney MDA Level

Antioxidant Parameters	LPO (nmol MDA/ mg protein)
	<i>R</i>
Proteins concentration (µg/mL)	-0.24
CAT (µmol H <sub>2</sub> O <sub>2</sub> consumed/min/mg protein)	-0.23*
SOD (U/mg of protein)	-0.24*
GPx (µmol GSH/mg protein/mL)	-0.24*
GST (nmol of CDNB conjugate formed min <sup>-1</sup> mg <sup>-1</sup> protein)	-0.16*
GSH (µmol/mg protein)	-0.47*
Urea (mmol/L)	0.30*
Creatinine (µmol/L)	0.34*
Daily body weight gain (gm)	-0.056*

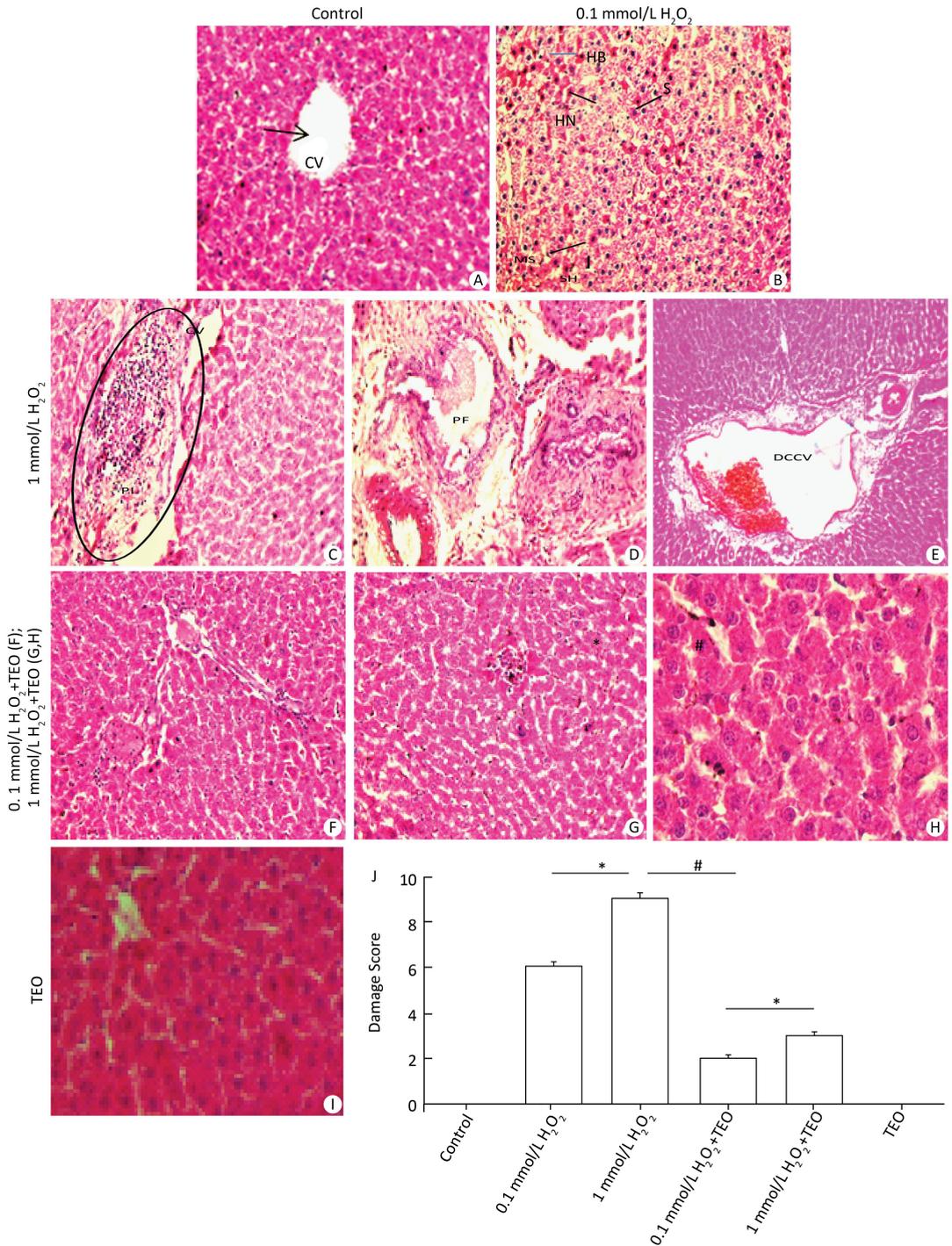
**Note.** \* Significant value was at *P*<0.05.

than did lower doses spaced more than a week apart. The liver showed loss of normal hepatocyte architecture, inflammatory cell infiltration, cytoplasmic vacuolization of liver cells, and disorganization of the hepatic parenchyma, the severity of which increased with increase in the dose. Furthermore, the oral administration of TEO revealed no histopathological changes to the liver and kidney compared with those in the untreated controls. The glomerulus and tubules appeared normal in the kidney tissue of the control rats (Figure 5). Low doses of H<sub>2</sub>O<sub>2</sub> induced mild vascular and inflammatory changes with signs of vascular congestion, tubular necrosis, and glomerular atrophy, which are a sign of toxicity. Proteinaceous casts in the lumen of renal tubules, decreased cell numbers (glomerulosclerosis), and disruption of the normal renal architecture were observed in the high-dose H<sub>2</sub>O<sub>2</sub>-treated group. Notably, histopathological analysis showed that TEO markedly alleviated injuries in the glomeruli and proximal tubules.

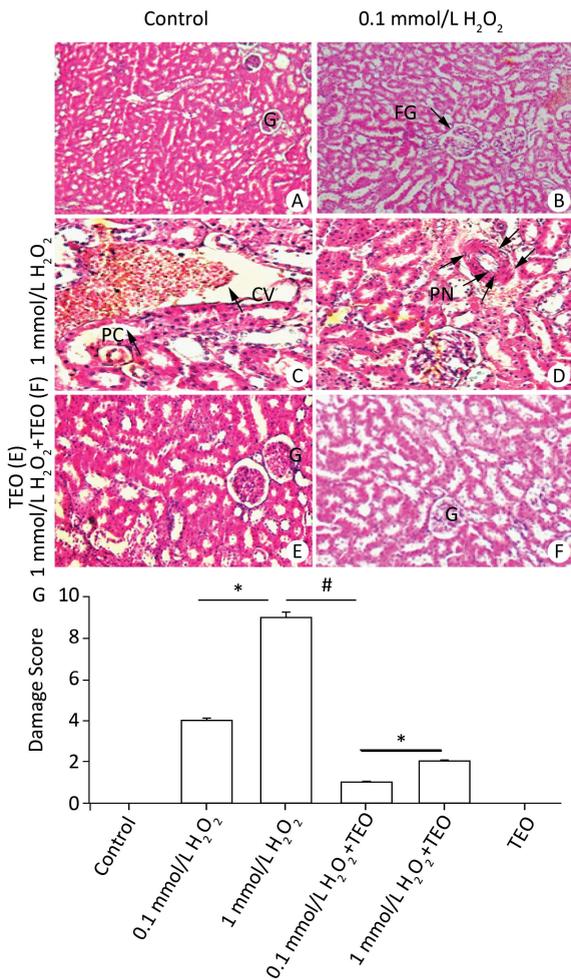
### DISCUSSION

Essentially, oxidative stress is an imbalance between the production of ROS and reactive nitrogen species (RNS) in the body, and has been considered an important etiological factor involved in various chronic diseases, such as atherosclerosis, arthritis, neurodegenerative diseases, diabetes mellitus, and cancer<sup>[24]</sup>. Oxidative damage also plays a critical role in different pathophysiological conditions including liver and kidney diseases. To prevent oxidative stress and harmful diseases, consumption of antioxidant-rich phytochemicals is recommended. Besides several antioxidant medications, the effects of safe and inexpensive natural compounds need to be evaluated. One of these natural phytochemicals is TEO, which has been reported to act as an effective antioxidant in reversing the cellular imbalance between ROS and RNS, thus preventing acute gastric injury in rats<sup>[10]</sup>. Therefore, in this study, we determined whether TEO has the potential to inhibit H<sub>2</sub>O<sub>2</sub>-induced hepatotoxicity and renal toxicity *in vivo*.

H<sub>2</sub>O<sub>2</sub> is a weak oxidizing agent that can directly inactivate some enzymes, usually by the oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and inside the cell, H<sub>2</sub>O<sub>2</sub> probably reacts with Fe<sup>2+</sup> or other redox metals such as Cu<sup>2+</sup> to form hydroxyl radicals, which may be the origin of many of its toxic effects<sup>[25]</sup>. H<sub>2</sub>O<sub>2</sub> can induce



**Figure 4.** (A): Histology of normal control rat liver. (B): Low dose treatment with H<sub>2</sub>O<sub>2</sub> (100 μmol/L). (C-E): High dose treatment with H<sub>2</sub>O<sub>2</sub> (1 mmol/L) showed development of portal-portal bridging fibrosis and portal inflammatory cells in rats with H<sub>2</sub>O<sub>2</sub>-induced hepatotoxicity. (F): TEO + low dose of H<sub>2</sub>O<sub>2</sub> (G,H): TEO+high dose of H<sub>2</sub>O<sub>2</sub> showed liver regeneration. (I): TEO. (J): Semi-quantitative analysis of H&E staining results. \**P*<0.001, #*P*<0.001. CV: central Vein; S: sinusoids; PF: portal fibrosis, PI: portal inflammation, HS: hepatocyte sheet, DCCV: dilated and congested central vein, HN: hepatocyte nuclei, NH: normal hepatocyte, L: lymphocyte, HB: hepatocyte ballooning, MS: microvesicular steatosis. Six rats were analyzed in each group. Magnification: A-D, F, G, and I, ×40; E, ×20; H, ×100.



**Figure 5.** Representative photomicrographs of H&E-stained sections of the kidneys of control and treated rats: control (A), LD H<sub>2</sub>O<sub>2</sub> (B), HD H<sub>2</sub>O<sub>2</sub> (C, D), TEO (E), TEO+high dose of H<sub>2</sub>O<sub>2</sub> (F). Glomerular basement membranes (arrows) were slightly thicker in HD H<sub>2</sub>O<sub>2</sub> group than those in the untreated rats at 2 weeks. (G): Semi-quantitative analysis of the H&E staining results. \**P*<0.001, #*P*<0.001. CV: congested vein; G: glomerulus; FG: fragmented glomeruli; PC: proteinaceous casts; PN: pycnotic nuclei. Six rats were analyzed in each group. Magnification: C-E, ×40; A, F, G, and I ×20; B, ×10; H, ×100.

OH<sup>-</sup> accumulation in liver and kidney cells by breaking down endogenous antioxidant defense mechanisms, and TEO effectively reduces H<sub>2</sub>O<sub>2</sub>-induced ROS production. The detrimental effects of H<sub>2</sub>O<sub>2</sub> on the liver and kidney in rats are strongly associated with biochemical changes, including impairment of function, metabolic

disorders, oxidative stress, and histological alterations. In the present study, higher serum aspartate aminotransferase and alanine aminotransferase activities, which generally serve as indicators of hepatocyte mitochondrial damage, were found in response to H<sub>2</sub>O<sub>2</sub> treatment. Thus, H<sub>2</sub>O<sub>2</sub>-induced oxidative stress increased enzymatic activity and subsequently altered the permeability of the liver cell membrane, resulting in the leakage of these enzymes into the circulation<sup>[26]</sup>. It was also reported that the release of transaminases (AST and ALT) and lactate dehydrogenase from the cell cytosol leads to cellular necrosis<sup>[27]</sup>, and these are therefore considered biomarkers for liver dysfunction. Of the two enzymes, ALT is considered the most liver-specific enzyme in rats because it is present in higher quantities in hepatocyte cytosol. Therefore, its presence in the serum is considered a marker of hepatocellular necrosis<sup>[28-29]</sup>. AST has also been found in cytosolic and mitochondrial forms, and is produced in the liver, as well as the kidney, pancreas, and erythrocytes of rats<sup>[30]</sup>. As we have observed, TEO has significant potential to attenuate H<sub>2</sub>O<sub>2</sub>-induced serum AST and ALT level elevations, and to function as an antioxidant in liver and kidney cells.

Oxidative stress affects many cellular functions through various mechanisms, including alteration of gene expression through transcriptional factor activation and alteration of protein expression levels, which are responsible for most cellular functional processes<sup>[31]</sup>. In the present study, we have observed that H<sub>2</sub>O<sub>2</sub> induced protein degradation in a dose-dependent manner, which was recovered by TEO treatment in liver and renal cells (Figure 2A and 3A). H<sub>2</sub>O<sub>2</sub> treatment increased lipid peroxidation through elevated hepatic and renal MDA levels, decreased hepatic and renal enzymatic levels (SOD, GST, GPx, and CAT), and decreased GSH antioxidant levels. MDA is a physiological ketoaldehyde produced by the peroxidative decomposition of unsaturated lipids as a by-product of arachidonate metabolism<sup>[32]</sup>. Direct treatment with hydrogen peroxide elevated serum levels of renal dysfunction markers such as urea, creatinine, and uric acid (Table 1). H<sub>2</sub>O<sub>2</sub>-induced the elevation of MDA levels, which was prevented by TEO pretreatment. MDA levels reflect the extent of cell damage due to oxidative stress<sup>[33]</sup>. It was also previously observed that the production of excess MDA due to tissue injury can combine with free amino acids and proteins, possibly altering their biological properties<sup>[31]</sup>.

Maintenance of intracellular redox homeostasis is dependent on a complex web of antioxidant molecules<sup>[34]</sup>, and a pivotal component is the tripeptide glutathione (g-L-glutamyl-L-cysteinylglycine), whose free thiol group in the cysteine residue is extremely reactive<sup>[35]</sup>. Moreover, the liver and kidney contain remarkably high levels of glutathione than those in other organs. Similarly, CAT is a tetramer of four polypeptide chain antioxidants found in nearly all living organisms exposed to oxygen. It is derived from the epididymis and seminal vesicle and detoxifies both intracellular and extracellular H<sub>2</sub>O<sub>2</sub> by reducing H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>, thus eliminating the potential ROS toxicity<sup>[36]</sup>. Oxidative stress conditions can be indicated by decreases in antioxidant enzyme levels (i.e. SOD, CAT, GPx, GST, and GSH), and this may be responsible for triggering cell apoptosis in rats. These findings suggest that a decrease in GSH may be used to monitor the severity and progress of hepatorenal toxicity. However, total GSH concentrations are high in these organs in healthy rats<sup>[37-38]</sup>. GSH peroxidase metabolizes H<sub>2</sub>O<sub>2</sub> in both cytosolic and mitochondrial compartments. In this context, GPx converts H<sub>2</sub>O<sub>2</sub> into water before it can produce ROS<sup>[39]</sup>. As we evaluated in our study, pre-administration of TEO prevented hepatorenal injuries and regulated serum hepatorenal biomarkers.

As per the histopathology of liver and kidney, oral administration of H<sub>2</sub>O<sub>2</sub> to rats for 2 weeks resulted in liver and kidney injury (Figure 4). However, the extent of injury in TEO pre-treated rats was significantly lower than that in the H<sub>2</sub>O<sub>2</sub>-treated groups. Liver and kidney injury was associated with elevated serum alanine aminotransferase and alkaline phosphatase levels, as well as pathological lesions in the liver<sup>[40]</sup>. It was also reported that oxidative stress associated with the formation of ROS plays an important role in the pathogenesis of hepatic injury<sup>[33]</sup> and renal failure<sup>[41]</sup>, which can affect all cells within the liver by inducing chronic inflammation, ischemia, apoptosis, and necrosis<sup>[42]</sup>. The histopathological results revealed that oral exposure to H<sub>2</sub>O<sub>2</sub> at different doses produced a remarkable dose-dependent damaging effect to both the liver and the kidney of rats, supporting our biochemical observations.

As the injurious effects of oxidative stress are counteracted by natural antioxidant defense mechanisms to protect the biological system against ROS, the protective effect of TEO against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress could be either direct

by inhibiting lipid peroxidation and scavenging free radicals or indirect through the enhancement of the activities of SOD and CAT, which act as intracellular free radical scavengers.

## CONCLUSIONS

We demonstrated that TEO is a potent antioxidant that was reported to ameliorate the effect of H<sub>2</sub>O<sub>2</sub>-induced hepatorenal toxicity through inhibition of lipid peroxidation, restoration of antioxidant enzyme activity, and alteration of serum biochemical parameters. This study helps to clarify the physiological changes caused by many known chemotherapeutic agents, pesticides, and drugs, the number of which has increased notably in the past few decades.

## CONFLICT OF INTEREST

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

## AUTHORS' CONTRIBUTIONS

GF conceived and designed the study; GF and BH performed the animal experiments and histological assessment; GF and AKT drafted the manuscript; GF performed the statistical analysis; and AKT revised the manuscript. All authors read and approved the final manuscript.

### Supplementary materials

The online version of this article contains supplementary materials (Table S1 and Figure S1).

### Abbreviations

ALT: Alanine aminotransferase  
 AST: Aspartate aminotransferase  
 CAT: Catalase  
 CDNB: 2,4-Dinitrochlorobenzene  
 DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid)  
 GSH: Reduced glutathione  
 GPx: Glutathione peroxidase  
 GSH: Glutathione  
 GSSG: Glutathione disulfide  
 GST: Glutathione S-transferase  
 HD: High dose  
 LD: Low dose  
 LPO: Lipid peroxidation  
 MDA: Malondialdehyde  
 ROS: Reactive oxygen species

RNS: Reactive nitrogen species

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

U: Unit

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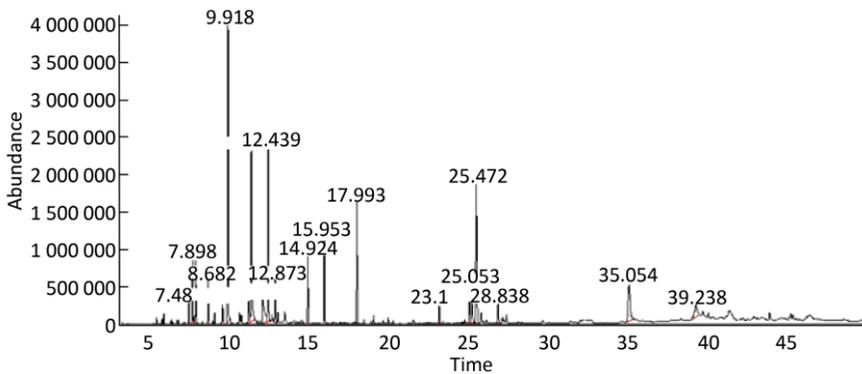
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**Table S1.** Chemical Composition of Essential Oils Extracted from *Thymus algeriensis* Using Analysis by GC-MS (Guesmi et al., 2014)

NO.	RT <sup>a</sup>	RI <sup>b</sup>	Components <sup>c</sup>	Peak Area (%)	
				TEO <sup>d</sup>	Identification <sup>e</sup>
1	7.481	1017	$\alpha$ -terpinene	1.18	GC-MS-RI <sup>f</sup>
2	7.705	1025	$p$ -cymene	3.22	GC-MS
3	7.899	1031	1,8-cineole	3.45	GC-MS
4	8.683	1060	$\gamma$ -terpinene	2.43	GC-MS-CAS <sup>g</sup> #
5	9.576	1089	Terpinolene	1.35	GC-MS-CAS#
6	9.919	1098	Linalool	18.05	GC-MS
7	11.378	1146	Camphor	13.03	GC-MS
8	12.431	1176	4-carvomenthenol	11.2	GC-MS
9	15.956	1289	Bornyl acetate	5.41	GC-MS
10	23.142	1511	$\gamma$ -cadinene	1.21	GC-MS
11	25.054	1578	Spathulenol	2.8	GC-MS
12	25.197	1582	Caryophyllene oxide	2.09	GC-MS
13	25.471	1590	Viridiflorol	11.71	GC-MS

**Note.** <sup>a</sup>RT (retention time); <sup>b</sup>RI (retention index) measured relative to *n*-alkanes (C<sub>10</sub>-C<sub>15</sub>); <sup>c</sup>Components listed in order of their retention index on HP-5MS column; <sup>d</sup>Plant codes; <sup>e</sup>GC-MS: identification based on a high match of mass spectra retention; <sup>f</sup>RI Identified by retention index and compared with those reported in the literature; <sup>g</sup>CAS # = Chemical Abstracts service reference number compared with those reported in the literature.



**Figure S1.** Chromatographic profile of TEO using gas chromatography coupled to a mass spectrometer (Guesmi et al., 2014). Guesmi F, Ben Farhat M, Mejri M, Landoulsi A. *In-vitro* assessment of antioxidant and antimicrobial activities of methanol extracts and essential oil of *Thymus hirtus* sp. *algeriensis*.