Protective effects of thymoquinone against acrylamide-induced liver, kidney and brain oxidative damage in rats

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Abstract

Acrylamide (AA), an industrial monomer, may cause multi-organ toxicity through induction of oxidative stress and inflammation. The antioxidant properties of thymoquinone (TQ), an active constituent of *Nigella sativa*, have been established before. The aim of the current study was to assess the protective effects of TQ against AA-induced toxicity in rats. Forty-eight male Wistar rats were divided into six groups each of eight rats. The first group acted as a negative control and received normal saline. Groups II and III were administered TQ orally at doses of 10 and 20 mg/kg b.wt., respectively, for 21 days. The four group received AA (20 mg/kg b.wt.) for 14 days. The five and six groups were given TQ at either dose for 21 days, starting seven days before AA supplementation (for 14 days). Acrylamide intoxication was associated with significant ($p < 0.05$) increases in serum levels of liver injury biomarkers (alanine transferase, aspartate transferase, and alkaline phosphatase), renal function products (urea, creatinine), DNA oxidative damage biomarker (8-oxo-2′-deoxyguanosine), and pro-inflammatory biomarkers (interleukin-1β, interleukin-6, and tumor necrosis factor-α). Moreover, AA intoxication was associated with increased lipid peroxidation and nitric oxide levels, while reduced glutathione concentration and activities of glutathione peroxidase, superoxide dismutase, and catalase in the liver, kidney, and brain. TQ administration normalized AA-induced changes in most serum parameters and enhanced the antioxidant capacity in the liver, kidney, and brain tissues in a dose-dependent manner. In conclusion, the current experiment showed that TQ exerted protective and antioxidant activities against AA-induced toxicity in mice.

Keywords Acrylamide · Thymoquinone · Antioxidant · Hepatotoxicity · Nephrotoxicity · Neurotoxicity

Introduction

Acrylamide (AA) is an industrial monomer, used in paper packaging, water treatment, and cosmetics (Yousef and El-Demerdash 2006). It is also recognized as a by-product of deep frying and has been described as a cooking-associated carcinogen due to its spontaneous formation during the cooking process of food (Acaroz et al. 2018). Acrylamide affects the nervous system and causes neurotoxic effects in both humans and animals. The main manifestations of AA neural toxicity in animals include hind limb foot splay, ataxia, skeletal muscle weakness, loss of sensation, and hypoactive

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reflexes (Pruser and Flynn 2011; Yousef and El-Demerdash 2006). Furthermore, animal experiments have shown neurotoxic, hepatotoxic, and nephrotoxic effects for AA (Aboubakr et al. 2018; Elkomy et al. 2018). These effects are attributed to impaired nitric oxide (NO) neurotransmission (Pruser and Flynn 2011), induced oxidative stress and lipid peroxidation (Yousef and El-Demerdash 2006; Zhu et al. 2008), and apoptosis (Mehri et al. 2012; Sumizawa and Igisu 2007). Besides, AA can cause DNA damage and may conjugate with hemoglobin and plasma proteins (Xie et al. 2008). The hepatic metabolism of AA produces both glicidiamine and glycidamide–DNA, which are more toxic than AA itself (Dybing et al. 2005). Testing protective agents, including natural antioxidants, against AA toxicity has been the focus of several pharmacological studies to date (Alturfan et al. 2012; Sumizawa and Igisu 2007, 2009).

Thymoquinone (TQ; 2-isopropyl-5-methyl-1,4-benzoquinone) is a phytochemical compound, obtained from *Nigella sativa* seeds. Prior studies on TQ showed various pharmacological activities, such as anti-ulcerogenic, antitumor, neuroprotective, and antioxidant effects (Ashraf et al. 2011; Nagi et al. 2010; Radad et al. 2009). The latter antioxidant effects are attributed to TQ free radical scavenging activity and increasing the activities of endogenous antioxidant enzymes such as glutathione peroxidase (GSH-Px), glutathione transferase (GST), superoxide dismutase (SOD), and catalase (CAT) (Badary et al. 2003). In addition, former investigations can protect the body tissues against the toxic effects of several xenobiotics, such as cisplatin (Badary et al. 1997), cyclophosphamide (Alenzi et al. 2010), ifosfamide (Badary 1999), and carbon tetrachloride (Khither et al. 2018).

To our knowledge, there is a paucity of data on the protective effects of TQ against AA toxicity in experimental studies. Therefore, the present study was therefore aimed to investigate the protective effects of TQ against AA-induced hepatic, renal, and cerebral oxidative damage in rats.

Materials and methods

Chemicals

Acrylamide in a pure form and TQ (CAS Number 490–91-5; molecular weight 164.20 g/mol; purity $\geq$ 98.5%) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA). Most of the kits, used in the current study, were purchased from Biodiagnostics Co. (Cairo, Egypt), except for ELISA kits that were purchased from R&D (Mannheim, Germany), 8-OhdG kits from Cayman Chemical Co. (Ann Arbor, MI, USA), and tumor necrosis factor-α from BioSource International Inc. (Camarillo, CA, USA). The absorbance of each marker was read using an automatic ELISA reader at 420 nm as per the manufacturer's instructions.

Experimental animals

A total number of 48 male rats (average body weight 150 to 250 g, average age 3–4 months) were used in the current study. Animals were obtained from the pharmaceutical department, laboratory animal unit, Faculty of Pharmacy, Beni-Suef University, Egypt. Over the experimental period, all animal handling, weighing, dosing, and slaughtering are carried out according to institutional animal care and use committee, Faculty of Veterinary Medicine, Beni-Suef University. Clean fresh water, supplied with standard diets and a protocol of 12-h dark and light cycle, was maintained throughout the experiment. The protocol of this study was started following a 7-day acclimation period.

Experimental design

Rats were divided into six equal groups. G1 rats (negative controls) received 1.5 ml/kg of saline oral gavage. G2 rats (positive controls) received AA at a daily dose of 20 mg/kg b.wt. (Lai et al. 2017) for 14 days p.o. G3 rats (TQ low dose) and G4 (TQ high dose) rats received TQ at a dose of 10 and 20 mg/kg. b.wt. p.o. for 21 days (Abdel-Daim et al. 2018; Alkharfy et al. 2015), respectively. G5 and G6 rats received AA for 14 days and TQ at 10 or 20 mg/kg b.wt. p.o., respectively; TQ administration started 7 days before AA administration, then both drugs were concurrently administered for 14 days (Fig. 1).

Collection and preparation of blood, serum and tissues

At the end of this study, rats were anesthetized through intraperitoneal injection of 1:1 xylazine: ketamine combination (0.1 ml/100 gm. b.wt.) after weighting and dose calculations. Immediately before anesthesia and decapitation, blood samples were collected from the medial canthus of the eye. After clotting, serum was obtained by centrifugation at 3000 for 15 min; serum samples were stored at – 20 °C until the analysis. After euthanasia, liver, kidney, and brain samples were collected and washed with normal saline 0.9%. For removal of red blood cells and clots, 50 mmol/l of ice-cold sodium phosphate buffer saline (100 mmol/l Na_2HPO_4/NaH_2PO_4) (pH 7.4), containing 0.1 mmol/l EDTA, was used. Later, tissue samples were grounded as 1 g of the tissue for every 5–10 ml of ice-cold buffer then centrifuged at 3000 rpm for 30 min. Then, the supernatants were kept at – 80 °C for later evaluation of the content and activities of antioxidant biomarkers and lipid peroxidation in each tissue type.

Serum biochemical analysis

Serum concentrations of urea, creatinine, and uric acid were evaluated as previously described by Coulombe and Favreau
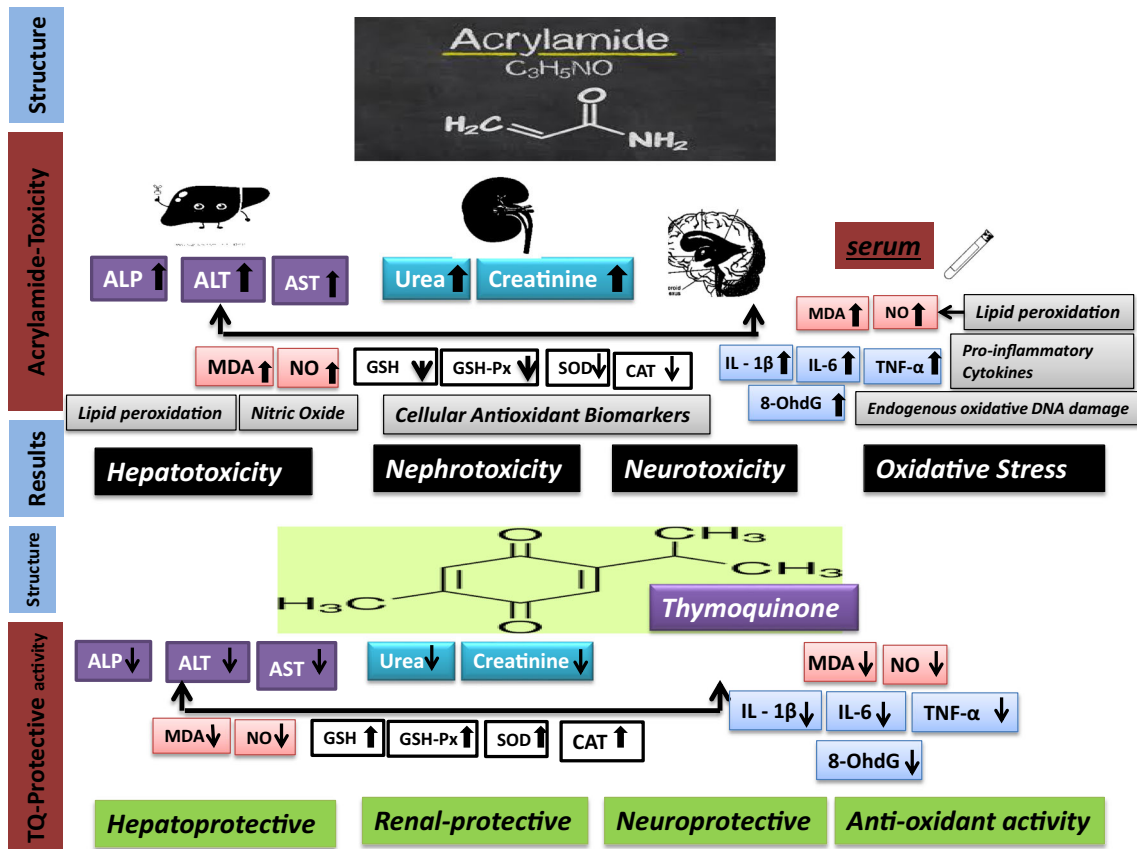
Fig. 1 Structure, toxicity, and protective effects of acrylamide and thymoquinone

(1963), Larsen (1972), and Whitehead et al. (1991), respectively. The serum concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated as per Reitman and Frankel (1957), while alkaline phosphatase (ALP) was estimated according to Tietz et al. (1983).

Assessment of tissue lipid peroxidation and antioxidant enzyme activities

The activities of the following antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), in the hepatic, renal, and cerebral tissues were estimated according to Aebi (1984), Nishikimi et al. (1972), and Paglia and Valentine (1967), respectively. While the tissue concentrations of reduced glutathione (GSH) were assessed as per Beutler (1963). Then, the tissue levels of malondialdehyde (MDA) and NO were assessed as per Uchiyama and Mihara (1978) and Green et al. (1982), respectively.

DNA oxidation and cytokines analysis

According to the manufacturer's instructions, an 8-OhdG competitive assay kit was used for measuring the serum levels of 8-OHdG, depending on both free 8-OHdG and DNA-

bound 8-OhdG assay. The serum concentrations of interleukin (IL)-1β, IL-6 (R&D, Mannheim, Germany), and TNF-α (BioSource International Inc., Camarillo, CA, USA) were estimated, using commercial ELISA kits according to the manufacturers' instructions.

Statistical analysis

Data analysis was conducted using the one-way analysis of variance (ANOVA) followed by Turkey's post hoc test for multiple comparisons using SPSS (version 20.0) software (IBM SPSS Statistic 20.0, Armonk, NY, USA). The results were expressed as mean ± standard error of mean (SEM). The p values lower than 0.05 were considered statistically significant.

Results

Serum biochemical analysis

Data analysis showed no significant differences between the negative control rats and those administered TQ alone at 10 and 20 mg/kg b.wt. except for the serum ALT level, which was significantly lower in the TQ20 group. Acrylamide oral administration to rats was associated with significant increases

Table 1 The effects of thymoquinone (TQ) administration on biochemical serum parameters in acrylamide (AA)-intoxicated rats
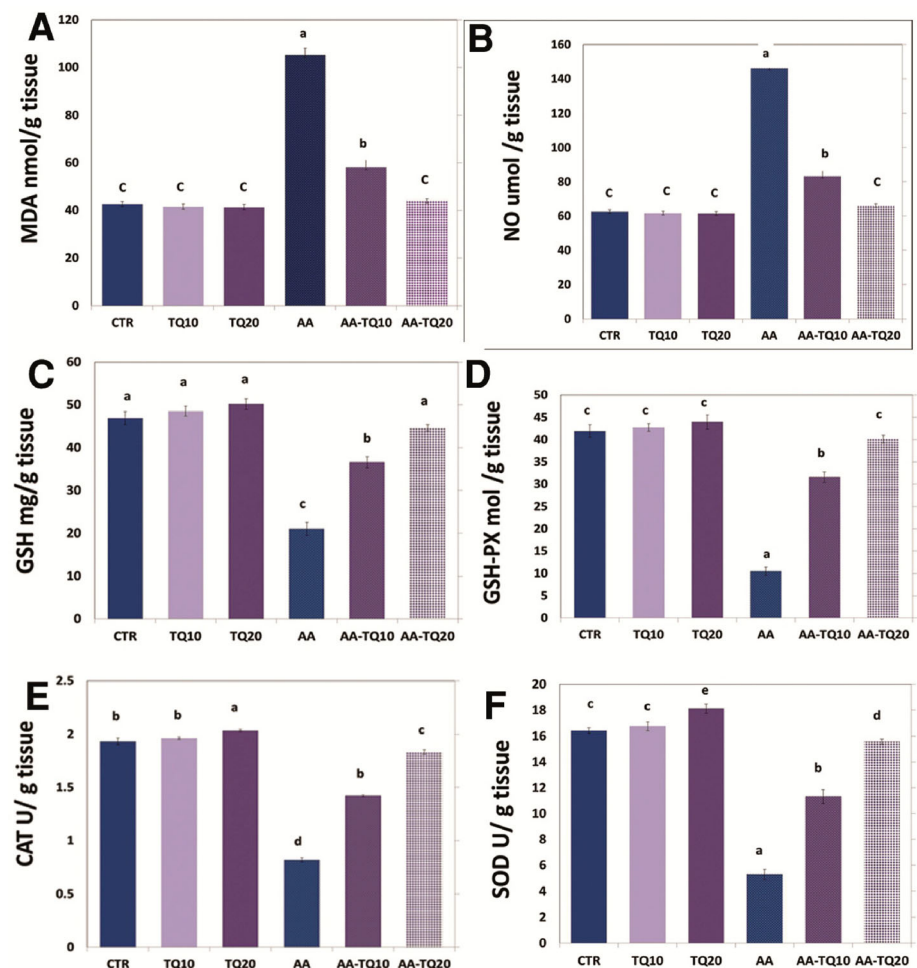
Group	AST (U/ml)	ALT (U/ml)	ALP (U/l)	Urea (mg/dl)	Creatinine (mg %)
Control	63.41 ± 1.11[c]	24.85 ± 0.64[d]	73.69 ± 1.46[c]	27.40 ± 1.12[d]	0.39 ± 0.24[c]
TQ10	62.43 ± 0.82[c]	23.37 ± 0.53[d]	70.21 ± 1.51[c]	25.94 ± 0.76[d]	0.40 ± 0.01[c]
TQ20	62.39 ± 1.24[c]	22.35 ± 0.32[e]	68.72 ± 1.36[c]	25.59 ± 0.63[d]	0.36 ± 0.01[c]
AA	117.24 ± 4.13[a]	52.75 ± 1.08[a]	144.18 ± 4.67[a]	82.82 ± 3.30[a]	4.24 ± 0.22[a]
AA-TQ10	72.91 ± 1.97[b]	36.76 ± 0.84[b]	91.46 ± 1.72[b]	50.33 ± 1.13[b]	1.23 ± 0.62[b]
AA-TQ20	65.37 ± 0.90[c]	27.20 ± 0.60[c]	75.28 ± 1.35[c]	35.62 ± 0.98[c]	0.57 ± 0.40[c]

Data are means ± SEM ($n = 8$ per group). Values with different superscript letters in the same column are significantly different at $p < 0.05$

AA, acrylamide; *ALP*, alkaline phosphatase; *ALT*, alanine transferase; *AST*, aspartate transferase; *TQ*, thymoquinone

in the serum levels of liver (ALT, AST, and ALP) and kidney (urea and creatinine) injury biomarkers ($p < 0.05$), compared with that to negative control rats. However, these elevations were significantly ($p < 0.05$) ameliorated after co-administration with both low and high doses of TQ (10 and 20 mg/kg b.wt.), yet serum levels remained significantly higher in the AA-TQ 10-mg/kg group, while they were restored to normal concentration ranges in the AA-TQ 20-mg/kg group (Table 1).
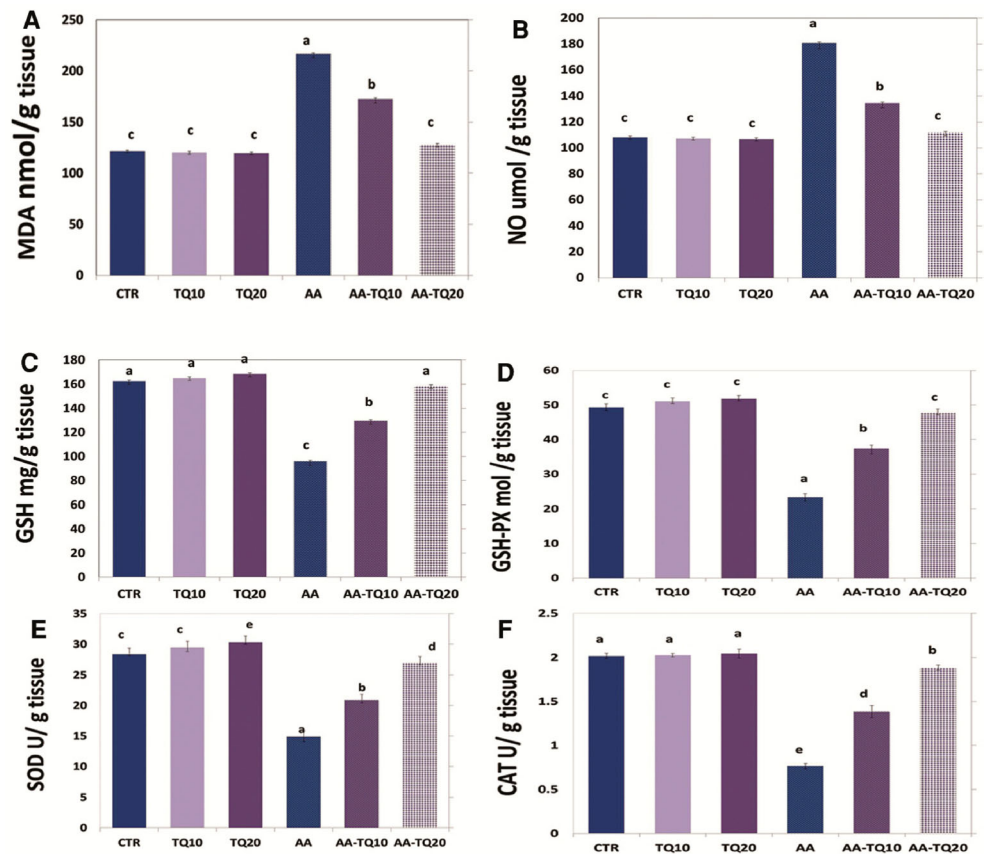
Hepatic lipid peroxidation and antioxidant status

Rats treated with TQ alone at 10 or 20 mg/kg b.wt. showed non-significant ($p > 0.05$) differences from the negative control group in all oxidative stress and antioxidant parameters except SOD and CAT hepatic tissue activities (which were significantly higher in the TQ20 group). The hepatic tissue levels of MDA and NO were significantly increased ($p < 0.05$) after AA treatment, while the hepatic tissue GSH

Fig. 2 The antioxidant effects of thymoquinone (TQ) against acrylamide (AA)-induced hepatotoxicity. MDA, malondialdehyde; NO, nitric oxide; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase. Data are presented as mean ± SEM. Columns labeled with different letters are significantly different ($p < 0.05$)

Fig. 3 The antioxidant effects of thymoquinone (TQ) against acrylamide (AA)-induced nephrotoxicity. MDA, malondialdehyde; NO, nitric oxide; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase. Data are presented as mean ± SEM. Columns labeled with different letters are significantly different ($p < 0.05$)

concentration and activities of GSH-Px, SOD, and CAT were significantly reduced in comparison with that in normal control rats. Acrylamide-intoxicated rats, previously treated with TQ, exhibited significantly lower hepatic tissue levels of MDA and NO ($p < 0.05$), as well as significantly higher GSH concentration and activities of antioxidant enzymes. Treatment with TQ at 20 mg/kg restored the normal concentration ranges of all tested hepatic parameters except SOD and CAT enzymatic activities (Fig. 2).

Renal lipid peroxidation and antioxidant status

Rats treated with TQ alone at 10 or 20 mg/kg b.wt. showed non-significant ($p > 0.05$) differences from the negative control group in all renal tissue oxidative stress and antioxidant parameters except SOD renal tissue activity (which was significantly higher in the TQ20 group). Intoxication with AA was associated with significant increases ($p > 0.05$) in renal tissue MDA and NO levels, as well as significant reductions of GSH concentration and activities of GSH-Px, SOD, and CAT, compared with that in negative control rats. Rats, administered TQ at 10 and 20 mg/kg b.wt. before AA intoxication, exhibited significant ameliorations ($p > 0.05$) of all AA-induced alterations with restorations of normal ranges of MDA, NO,

GSH concentrations and GSH-Px activity in the AA-TQ 20-mg/kg group (Fig. 3).

Brain lipid peroxidation and antioxidant status

Rats treated with TQ alone at 10 or 20 mg/kg b.wt. showed non-significant ($p > 0.05$) differences from the negative control group in all oxidative stress and antioxidant parameters except SOD and CAT cerebral tissue activities. Acrylamide intoxication increased ($p < 0.05$) the brain tissue levels of MDA and NO while reduced the GSH concentration of GSH and activities of GSH-Px, SOD, and CAT. Co-administration of TQ at 10 and 20 mg/kg b.wt. significantly ($p < 0.05$) ameliorated all AA-induced alterations with restorations of normal ranges of MDA, NO, GSH concentrations and GSH-Px activity in the AA-TQ 20-mg/kg group (Fig. 4).

Inflammatory cytokines and DNA oxidation marker

Non-significant ($p > 0.05$) variations were observed between groups administered TQ at 10 and 20 mg/kg b.wt. and the normal control rats. Acrylamide administration significantly ($p < 0.05$) increased the serum cytokines and 8-OhdG levels in comparison with that in the negative controls. TQ at doses of

Fig. 4 The antioxidant effects of thymoquinone (TQ) against acrylamide (AA)-induced neurotoxicity. MDA, malondialdehyde; NO, nitric oxide; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase. Data are presented as mean ± SEM. Columns labeled with different letters are significantly different ($p < 0.05$)
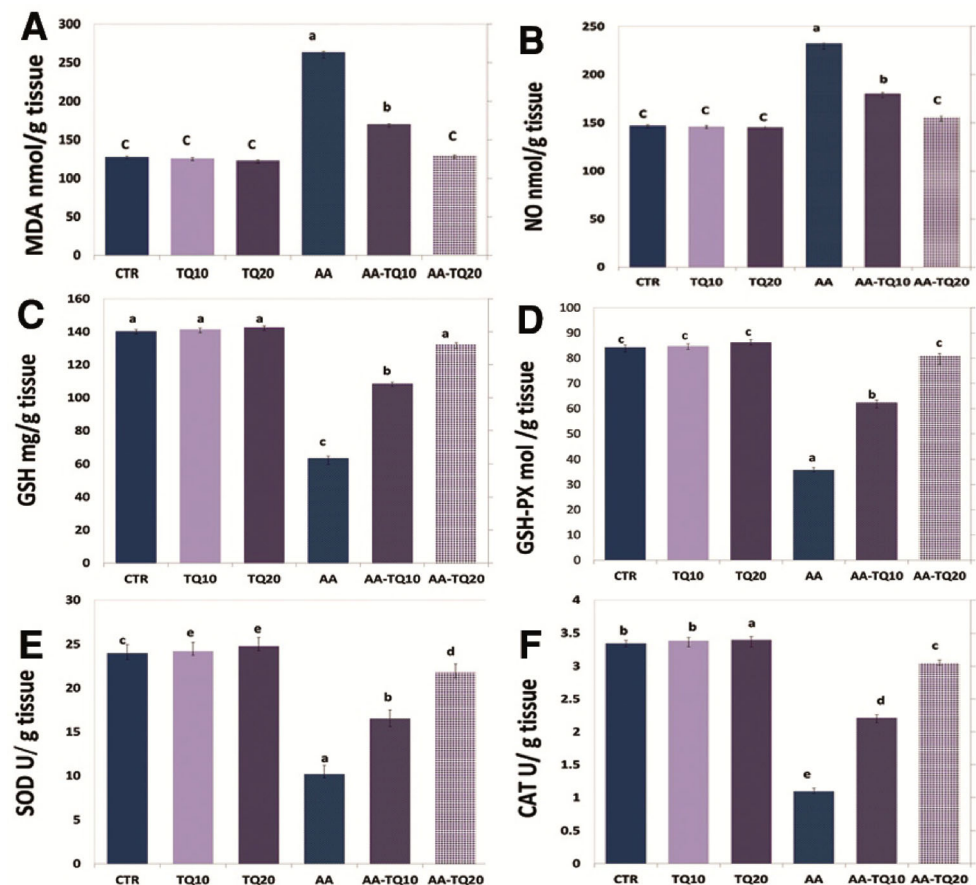
10 and 20 mg/kg b.wt. significantly ($p < 0.05$) decreased IL-1β, IL-6, TNF-α, and 8-OhdG serum concentrations, compared with that in the AA group alone with restoration of normal cytokine concentration in the AA-TQ 20-mg/kg group (Fig. 5).

Discussion

In the present study, administration of AA at a dose of 20 mg/kg b.wt. for 14 days in rats was associated with marked hepatorenal injury, oxidative stress and DNA damage, and increased serum levels of pro-inflammatory cytokines. Furthermore, the study documented the protective effects of TQ against AA toxicity at two different doses (10 and 20 mg/kg b.wt. p.o.) as evidenced by reduced hepatic injury and renal function biomarkers levels in the serum of AA-intoxicated animals, as well as improved oxidative balance in the hepatic, renal, and cerebral tissues of rats.

Reactive oxygen species (ROS) are produced normally inside the body; however, their levels are maintained balanced through internal antioxidant mechanisms (Abdel-Daim 2014). Exposure of animals or humans to any xenobiotic or

hazardous materials causes an imbalance in ROS production and neutralization (Abdel-Daim et al. 2013). Such oxidative stress is implicated in multiple diseases, such as atherosclerosis, cancers, and diabetes (Abdel-Daim et al. 2010a, b). In the current study, AA (an industrial xenobiotic) caused marked oxidative stress in different rat tissues (Zhang et al. 2013). These results are in line with previous studies about AA (Alturfan et al. 2012; Xie et al. 2008; Zhang et al. 2013). This can be explained by reducing the endogenous antioxidant capacity (Salvemini et al. 2002). Moreover, AA binds with GSH, which is essential for removing oxygen free radicals (Pradeep et al. 2007), which results in ROS accumulation and damage to cellular macromolecules (Beckman and Koppenol 1996). Besides lipid peroxidation, oxidative stress increases the expression of pro-inflammatory cytokines as IL-1β and TNF-α, explaining the interplay between oxidative stress and inflammation (Sayed 2008; Sayed and Morcos 2007).

Data obtained in this study confirmed the liver and kidney injury induced via AA intoxication. The significant increases in liver and kidney serum injury markers are in accordance with previous studies on experimental AA toxicity (Alturfan et al. 2012; Zhang et al. 2013). The increase of serum ALT, AST, and ALP levels are probably due to hepatocyte injury

Fig. 5 The protective effect of thymoquinone (TQ) against acrylamide (AA)-induced changes in the serum levels of 8-OHdG (A), TNF-α (B), IL-1β (C), and IL-6 (D) in rats. Data are presented as mean ± SEM
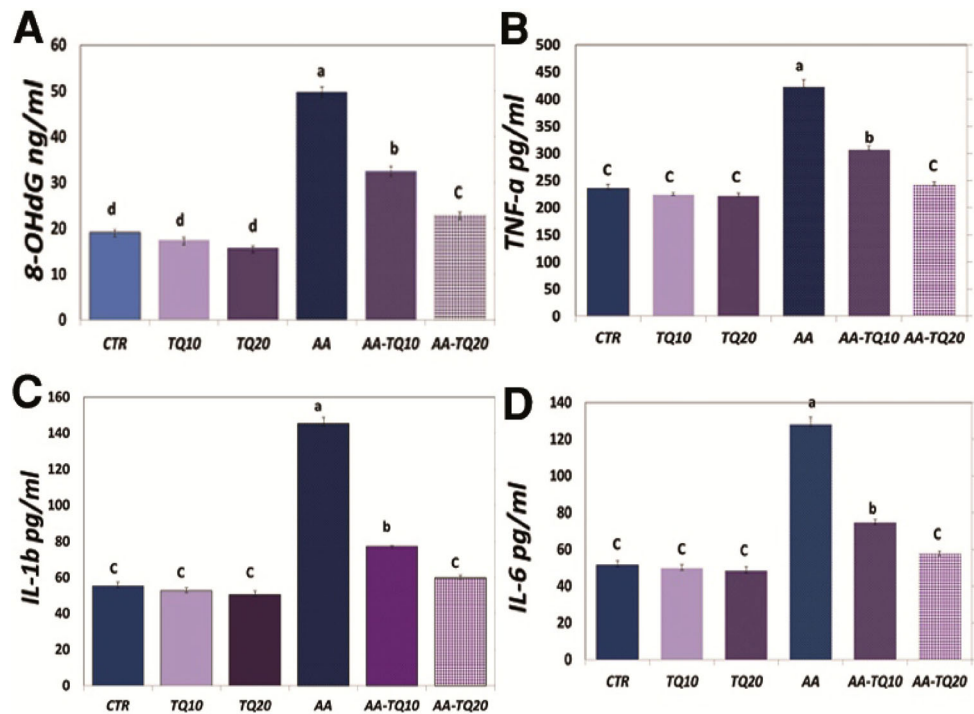
and leakage of these enzymes to the circulation, While the increases in serum creatinine and urea may be due to the degradation of pyrimidines and purines (DNA breakdown), also confirmed by increasedserum levels of (8-OhdG) and worsening renal functions.

The current results indicated that TQ normalized the serum concentrations of inflammatory cytokines, DNA oxidation marker, and renal and hepatic injury biomarkers, besides reducing oxidative stress and lipid peroxidation in hepatic, renal, and cerebral tissues. Thymoquinone, the main constituent in *Nigella sativa*, exhibited antioxidative effects in several prior experiments (Hosseinzadeh et al. 2007; Kanter et al. 2006). In its reduced form (thymohydroquinone), it acts as an electron donor to hydroxyl radicals (OH^{-1}) and superoxide radicals, which attack polyunsaturated fatty acids in the cell membrane. This explains the potent free radical scavenging capacity of TQ (Badary et al. 2003; Khither et al. 2018; Staniek and Gille 2010). Moreover, TQ has been shown to suppress the expression of inducible nitric oxide synthase (iNOS), which synthesizes NO (El-Mahmoudy et al. 2002); however, it suppresses the expression of CAT and glutathione-S-transferase (Ismail et al. 2010). In a former study, the antioxidant capacity of TQ was superior to that of the established antioxidant (ascorbic acid) (Staniek and Gille 2010).

Furthermore, TQ showed protective effects against AA toxicity in the hepatic, renal, and cerebral tissues. This comes in accordance with previous studies about the protective effects of TQ on hepatocytes against carbon tetrachloride

(Khither et al. 2018) and cyclophosphamide (Alenzi et al. 2010). Moreover, TQ has been shown to protect the kidneys against cisplatin (Badary et al. 1997) and ifosfamide (Badary 1999) and the brain against the neurotoxic effects of irradiation (Ahlatci et al. 2014) and toluene exposure (Kanter 2008). Another interesting finding of the current study is the TQ ability to reduce the inflammatory biomarkers in this serum, which may be either a direct effect on their expression levels of due to mitigation of oxidative stress (Bargi et al. 2017; Firdaus et al. 2018; Uemura et al. 2010).

In conclusion, the present study showed that AA intoxication induces hepatorenal injuries via oxidative stress, lipid peroxidation, DNA oxidative damage, and inflammatory response. Moreover, oral TQ administration at 10 and 20 mg/kg b.wt. protected the mice tissues from AA-induced oxidative stress in a dose-dependent manner.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations AA, acrylamide; TQ, thymoquinone; ALT, alanine transferase; ALP, alkaline phosphatase; AST, aspartate transferase; MDA, malondialdehyde; NO, nitric oxide; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; IL, interleukin; TNF, tumor necrosis factor

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