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Protective role of thymoquinone against acrylamide-induced nephrotoxicity in rats

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ABSTRACT

The goal of this study was to see how thymoquinone (TQ) altered acrylamide (ACR) induced toxicity in rats. The current study was carried out on 28 male Wistar rats divided into 4 experimental groups: control (saline, once daily, PO); TQ group (20 mg/kg, once daily, PO); ACR group (20 mg/kg, once daily, PO). The ACR+TQ group received ACR, TQ as mentioned. Saline, TQ and/or ACR, were administered for 28 days. The serum was separated, and the kidney tissues were collected, in order to assess biochemical and oxidative stress markers, as well as histological alterations. Levels of creatinine and urea were increased while total protein and albumin were decreased significantly in ACR-treated groups when compared to control. The concentration of malondialdehyde (MDA) in the kidney tissues of the ACR-treated groups were notably substantially higher than in the other groups. Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), and reduced glutathione (GSH) levels in renal tissues were significantly lowered in the ACR-treated groups compared to the other groups. Furthermore, histological kidney lesions indicated statistically significant differences between the groups that received ACR and the control groups. Controlling ACR-induced oxidative stress, in particular through the use of TQ, plays a crucial role in kidney protection.

1. INTRODUCTION

Acrylamide (ACR), a white odorless water-soluble crystal, (Kahkeshani et al., 2015) used in drinking water treatment and the removal of wastewater suspended solids. Additionally, it is employed in the industry of cosmetics, mining, and the preparation of waterproofing agents for dams and subways (Pennisi et al., 2013). Moreover, Acaroz et al. (2018) reported that ACR is a food carcinogenic agent that is produced during the high temperature or frying cooking of food. The most accused foods that are associated with ACR health hazards in humans are fried cereals, bread, and potato (Guyton and Hall, 2006). The most routes of administration of ACR are through the gastrointestinal tract, respiratory tract, and skin contact and the hepatic biotransformation process of it resulted in the formation of glycidamide (Szczerbina et al., 2008) and glycidamide-DNA (Dybing et al., 2005). Glyciamide and Glyciamide-DNA are more toxic than ACR. Moreover, ACR and its metabolite, glycidaimde, are considered carcinogenic agents that affect different organs in the body as, the liver, kidney, brain, lungs, and intestine (Capuano and Fogliano, 2011; Bin-Jumah et al., 2021). Hepatotoxicity, nephrotoxicity, and neurotoxicity induced by ACR were described in several reports (Yousef and El-Demerdash, 2006; Zhu et al., 2008; Aboubakr et al., 2018; Elkomy et al., 2018). The main mechanism of action is the induced ACR oxidative stress and lipid peroxidation as reported by several studies (Abdel-Daim et al., 2020; Elhelaly et al., 2019). Moreover, Pruser and Flynn (2011) concluded that the toxic effects of ACR occurred as a result of diminished nitric oxide (NO) neurotransmission. Besides, ACR-induced apoptosis (Mehri et al. 2012), triggered DNA impairment and may conjugate with plasma proteins and hemoglobin (Xie et al., 2008).

Nigella sativa, Black cumin, is a natural herb that used in alternative medicine for treating different types of diseases in human beings for hundreds of years ago. The most active and powerful constituent of the *Nigella Sativa* oil is thymoquinone (TQ). Several experiments reported the different protective and ameliorative effects of N. Sativa and its TQ constituent against some hazards including kidney and liver toxicity, inflammation, bacterial infection, depression, oxidative stress, carcinogenicity, and tumor formation (Radad et al., 2009; Nagi et al., 2010; Ashraf et al., 2011). In addition, Badary et al. (2003) explained the antioxidant effect of TQ in their study through the increased activities of antioxidant enzymes such as CAT, SOD, GPx, and GST. Furthermore, in the former toxicological studies, TQ exerted a protective effect against some toxicants like malathion inhalation (Abdo et al., 2021), carbon tetrachloride (Khither et al., 2018), cisplatin (Badary et al., 1997), bisphenol (Abdel-Wahab, 2014; Fadishei et al., 2021), ifosfamide (Badary, 1999), and cyclophosphamide (Alenzi et al., 2010). Moreover, *Nigella sativa* and TQ protect the renal tissues against multiple types of xenobiotics such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (Erdemli et al., 2020), amikacin (Ozer et al., 2020), doxorubicin (Kaymak et al., 2022), arsenic (Sener et al., 2016), and fipronil (Abdel-

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Daim et al., 2018). Therefore, according to the nephroprotective beneficial effects of TQ which were approved by the previous studies, in the current study, we attempted to determine whether the TQ would reduce the ACR-induced renal toxicity in adult male albino rats.

2. MATERIAL AND METHODS

2.1. Chemicals

ACR and TQ were obtained from Sigma Chemical Company (St. Louis, Mo, USA) and stored at room temperature till use. The analytical kits (Bio-diagnostics Co, Giza, Egypt).

2.2. Experimental design

Twenty-eight Wister Albino male rats weighing160±30 g (Animal House, Fac Vet Med, Benha University, Egypt). Rats were maintained at 25°C, and subjected to a light / dark cycle (12 / 12 h) with freely watering and feeding on a commercial pellet diet. The animals were left for 7 days before the beginning of the study. The rats were randomly divided into 4 groups of seven rats each, as shown: control group (saline once daily, PO); TQ group (20 mg/kg once daily, PO; Abdel-Daim et al., 2020); ACR group (20 mg/kg once daily, PO; Rahangadale et al., 2012); and ACR+TQ group (dosage as mentioned above). All treatments were administered for 28 days.

2.3. Blood, serum, and tissues collection and preparation

One day after the last dose, rats were anesthetized and blood samples were obtained from the retro-orbital plexus in plain tubes (serum was obtained by centrifugation at 1200 g for 15 min). Serum was stored at −20°C for biochemical analysis. Kidneys were removed, washed with 0.9% NaCl plus distilled water, and perfused with ice-cold 50 mmol/L sodium phosphate-buffered saline containing 0.1 mmol/L EDTA. Then the renal tissue specimens were stored at -80^{O}C until used. These specimens were homogenized on ice (Electrical Homogenizer) using 1g tissue plus 5 ml phosphate buffer (pH 7.4). The homogenates were centrifuged for 20 min at 1200 xg for supernatant separation. The collected supernatant samples were used for oxidative stress biomarkers detection.

2.4. Serum biochemical analysis

Serum urea and creatinine concentrations were estimated according to Coulombe and Favreau (1963), and Larsen (1972), respectively. Serum total protein concentration was estimated by the technique described by Koller (1984). Serum albumin concentration was estimated according to Doumas et al. (1971).

2.5. Oxidative cascade and lipid peroxidation analysis

The antioxidant enzymatic activities for CAT, SOD, and GSH-Px in the kidney tissues were estimated according to the techniques of Aebi (1984), Nishikimi et al. (1972), and Paglia and Valentine (1967), respectively. While the GSH renal tissue concentrations were evaluated according to Beutler (1963). Also, the renal tissue concentration of the lipid peroxidation indicator, MDA, was estimated according to Uchiyama and Mihara (1978).

2.6. Histopathology study

The remaining kidney tissues were preserved for 24 h in 10% neutral buffered formalin for histopathological analysis. Following that, the renal tissue samples were washed with tap water before being immersed in ethyl alcohol serial dilutions. The samples were then immersed in paraffin and cut into 4 m thick pieces. Under a light microscope, hematoxylin and eosin were used for staining of the cut sections for histological investigation (Bancroft and Cook, 1994).

2.7. Immunohistochemical examination

Immunohistochemistry Bax, Bcl-2, and caspase 3 Paraffin-embedded tissue sections of 3 µm thickness were rehydrated in xylene and then rehydrated by utilizing graded ethanol solutions. Slides were then inactivated with 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 2 h. After that, sections were immunostained with one of the following primary antibodies; rabbit polyclonal anti-Bax antibody (GeneTex Inc., USA), and rabbit polyclonal anti-Bcl-2 antibody (GeneTex Inc., USA), rabbit polyclonal anti caspase 3 antibody (GeneTex Inc., USA) at a concentration of 1 µg/ml compromising 5% BSA in TBS and left incubated at a temperature of 4 °C overnight. The slides were rinsed by TBS, the sections were incubated with goat anti-rabbit secondary antibody. Sections were washed with TBS and incubated in a solution of diaminobenzidine (0.02%) containing 0.01% H2O2 for 10 min. Counterstaining was conducted by utilizing hematoxylin, and the slides were investigated under a light microscope.

2.8. Statistical analysis

Data are displayed as mean±SE. Using the statistical software package SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA) data were analyzed using one-way ANOVA followed by Duncan's post hoc test for multiple group comparisons.

3. RESULTS

TQ had a marked influence on levels of kidney indices. ACR exerted a considerable elevation in the serum urea and creatinine levels. Treatment with TQ significantly declined the serum levels of urea and creatinine when compared to ACR treated group (Figure 1). Also, the serum concentrations of the total protein and albumin were significantly reduced in ACR treated group compared to the control group. While in ACR+TQ treated group, total protein and albumin concentrations were increased compared to ACR treated group and these results were shown in Figure (1).

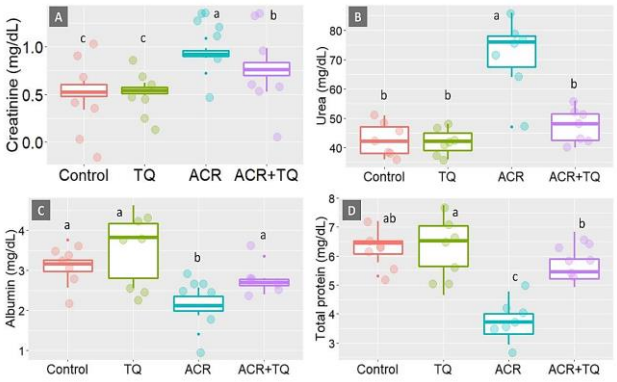

Figure (1): Boxplots of creatinine (A), urea (B), albumin (C), and total protein (D) with whiskers bar and median line of Control, thymoquinone (TQ), acrylamide (ACR), and ACR+TQ groups. Values are proffered as mean ± SD (n = 7).

ACR treatment caused oxidative damage in the renal tissue, as evidenced by a considerable rise in MDA levels, and decreased SOD, CAT activities, GSH, and GSHpx levels. Treatment with TQ caused marked mitigation of these parameters towards the control (Figure2). Also, Histopathology supported these findings.

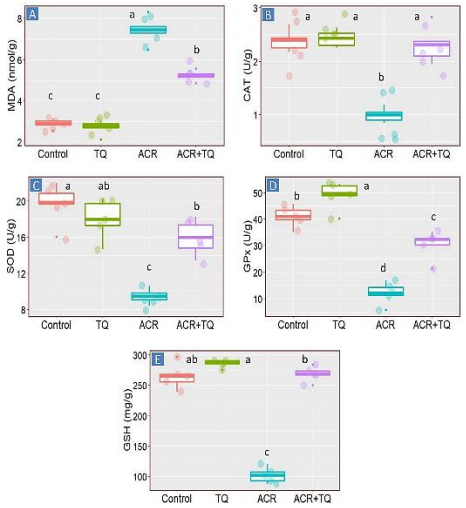

Figure (2): Boxplot of MDA (A), CAT (B), SOD (C), GPx (D), and GSH (E) with whiskers bar and median line of Control, thymoquinone (TQ), acrylamide (ACR), and ACR+TQ groups. Values are proffered as mean ± SD (n = 7).

The kidney of the control and TQ groups showed normal histology in which the nephron consists of the glomerulus (G) and the renal tubule (RT) (Figure 3, A-B). ACR group showed multifocal acute tubular necrosis infiltrated with mononuclear cell infiltration (arrows) (Figure 3 C).
ACR+TQ group showed normal histology in which the nephron consists of the glomerulus (G) and the renal tubule (RT) (Figure 3 D).

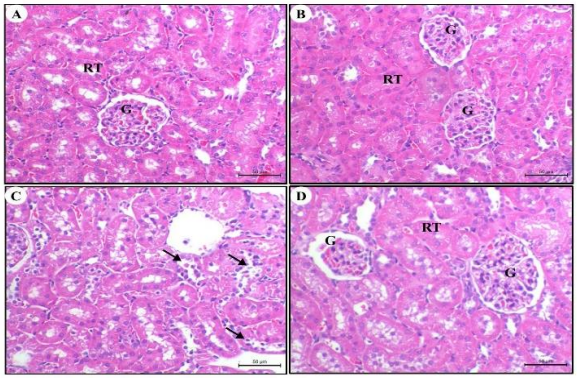

Figure 3 Photomicrograph of kidney stained with H&E of control group (Figure 3 A), showing normal histology in which, the nephron consists of the glomerulus (G) and the renal tubule (RT). TQ group (Figure 3 B) showed normal histology in which the nephron consists of the glomerulus (G) and the renal tubule (RT). ACR group (Figure 3 C) showed multifocal acute tubular necrosis infiltrated with mononuclear cells infiltration (arrows). ACR+TQ group (Figure 3 D) showed normal histology in which the nephron consists of the glomerulus (G) and the renal tubule (RT)

The Bax immunostaining of the control and TQ groups showed mild immunostaining of the Bax antibody within the renal tubular epithelium. TQ group (Figure 4 A- B). ACR group showed marked cytoplasmic and nuclear expression of Bax antibody within the renal tubular epithelium (Figure 4 C). ACR+TQ group showed a marked decrease in Bax antibody expression within the renal tubular epithelium, (Figure 4 D). The Bcl2 immunostaining of the control and TQ groups showed marked cytoplasmic expression of the Bcl2 antibody within the renal tubular epithelium (Figure 5 A-B). ACR group showed marked a decrease in the expression of Bcl2 antibody within the renal tubular epithelium (Figure 5 C). ACR+TQ group showed marked increased expression of Bcl2 within the renal tissues (Figure 5 D). The caspase 3 immunostaining of the control and TQ groups showed scanty expression of caspase 3 antibody within the renal tubular epithelium (Figure 6 A-B). ACR group showed marked expression of caspase 3 (cytoplasmic and nuclear) within the renal tubular epithelium (Figure 6 C). ACR+TQ group showed a marked decrease in caspase 3 expression within the renal tubular epithelium (Figure 6 D).

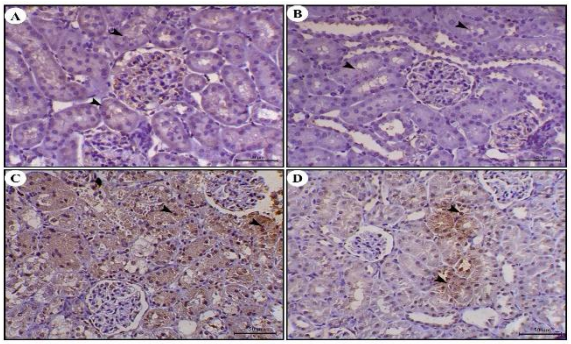

Figure (4): Effect of ACR and/or TQ on Bax expression in the kidney. Kidney of the control group (Figure 4 A) showed mild immunostaining of Bax antibody within the renal tubular epithelium (arrowhead). TQ group (Figure 4 B) showed mild immunostaining of Bax antibody within the renal tubular epithelium (arrowhead). ACR group (Figure 4 C) showed marked cytoplasmic and nuclear expression of Bax antibody within the renal tubular epithelium (arrowhead). ACR+TQ group (Figure 4 D) showed a marked decrease in Bax antibody expression within the renal tubular epithelium (arrowheads), Bax IHC, X200, bar= 50 µm

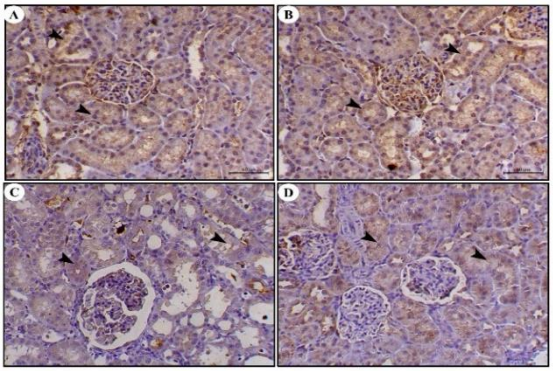

Figure (5): Effect of ACR and/or TQ on Bcl2 expression in the kidney. Kidney of the control group (Figure 5 A) showed marked cytoplasmic expression of Bcl2 antibody within the renal tubular epithelium (arrowheads). TQ group (Figure 5 B) showed marked cytoplasmic expression of Bcl2 antibody within the renal tubular epithelium (arrowheads). ACR group (Figure 5 C) showed a marked decrease in the expression of Bcl2 antibody within the renal tubular epithelium (arrowheads). ACR+TQ group (Figure 5 D) showed marked increased expression of Bcl2 within the renal tissues (arrowheads), bars = 50 µm.

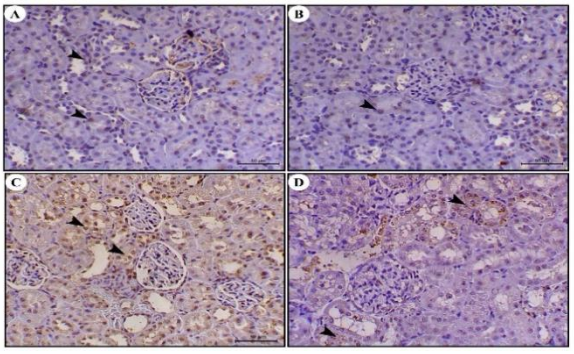

Figure (6): Effect of ACR and/or TQ on Caspase 3 expression in the kidney. Kidney of the control group (Figure 6 A) showed scanty expression of caspase 3 antibody within the renal tubular epithelium (arrowheads). TQ group (Figure 6 B) showed scanty expression of caspase 3 antibody within the renal tubular epithelium (arrowheads). ACR group (Figure 6 C) marked expression of caspase 3 (cytoplasmic and nuclear) within the renal tubular epithelium (arrowheads). ACR+TQ group (Figure 6 D) showed a marked decrease of caspase 3 expression within the renal tubular epithelium (arrowheads), bars = 50 µm.

4- DISCUSSION

The current study was investigated to estimate the protective role of TQ against ACR-induced renal tissue damage. Our

results exhibited that the administration of ACR markedly elevated urea and creatinine levels. This is consistent with previous data which showed deteriorations in renal function after the acute administration of ACR (Abdel-Daim et al., 2020). Conversely, TQ groups exposed a marked decreasing in both parameters. These findings are in harmony with an earlier study which reported that TQ administration of ACR-treated rats markedly ameliorated ACR-induced kidney impairment and lowered urea and creatinine levels (Abdel-Daim et al., 2020; Mostafa et al., 2021). The increase of ROS production over the normal levels during the cellular metabolism is an indicator of the abnormal conditions like ACR toxicity that leads to oxidative stress. Nita and Grzybowski, (2016) reported that DNA deterioration and amino acids oxidation as a result of oxidative stress. Furthermore, Abdel-Daim et al. (2015) concluded that the production of ROS and oxidative stress interrupt cellular function and caused multiple pathological syndromes. In our study, serum protein and albumin levels were reduced in ACR-treated rats compared with the control group. These results are attributed to the changes and delaying in protein metabolism and synthesis. In spite of the fact that ACR may cause abnormal gene expression that caused significant reductions in blood globulin levels (Mahmood and Amin, 2015). Lipid peroxidation in the renal tissue in the findings of the current study was proved by the elevation of MDA concentration in the ACR-treated rat. ACR disrupts the function of the mitochondria and increases ROS production leading to lipid peroxidation (Liu et al., 2015). The enzymatic antioxidants including CAT, SOD, and GSH-Px protect the body tissues against oxidative stress. Also, GSH is known as the first line of defense against oxidative damage disorders caused by environmental hazards as reported by Pizzino et al. (2017). In the current experiment, the ACR-intoxicated group showed a marked reduction in CAT, SOD, and GSH-Px activities and GSH levels in the renal tissue homogenates. Previous studies reported that the concentration of the GSH increased in ACR toxicity to overcome the damage oxidative effects and this occurred with the subsequent overproduction of the free radicals, NO, and superoxide (Yousef and El-Demerdash, 2006; Pradeep et al., 2007)

Group treated with ACR+TQ showed a remarkable reduction in MDA and marked elevation in GSH-Px, SOD, and CAT activities and GSH level in renal homogenates. The same results were noted by another study, which reported that TQ pretreatment attenuated these parameters when given in combination with ACR (Abdel-Daim et al., 2020). TQ in the previous study has the ability to keep normal cell membrane integrity against ACR- induced oxidative damage by increasing the antioxidant enzymatic activities and reducing MDA and free radicals' concentrations. (Abdel-Daim et al., 2020). Conversely, TQ groups exposed a marked decrease in both parameters. These findings are in harmony with an earlier study which reported that TQ administration of ACR-treated rats markedly ameliorated ACR-induced kidney impairment and lowered urea and creatinine levels (Abdel-Daim et al., 2018; Mostafa et al., 2021).

Furthermore, the data in the current study are documented by previous studies reported by other investigators (Aycan et al., 2014; Shaterzadeh-Yazdi et al., 2018; Abdel-Daim et al., 2019; Guo et al., 2020; Akgül et al., 2021; Khalifa et al., 2021; Landucci et al., 2021). They reported different mechanisms of action of TQ as antioxidant, anti-inflammatory, anti-apoptotic, and immunomodulatory effects.

In this study, ACR upregulated Bax and caspase 3 and downregulated Bcl-2 expression. The proteins Bax and Bcl-2 play important roles, with Bax acting as a pro-apoptotic factor and Bcl-2 acting as an anti-apoptotic factor. Furthermore, TNF- activation causes oxidative stress and renal inflammation (Abouzed et al. 2021). In this study, ACR upregulated Bax and caspase 3 and downregulated Bcl-2 expression. This suggests that ACR treatment can cause inflammation and apoptosis in renal tissues, followed by necrosis. TQ treatment significantly attenuated the immunohistochemical changes in the renal tissue induced by exposure to ACR. The possible mechanism of TQ is attributed to its antioxidant and anti-inflammatory properties. TQ can suppress the expression of iNOS, CAT, and GST (El-Mahmoudy et al. 2002, Ismail et al. 2010).

Histopathologically, the renal tissues of the ACR group showed multifocal acute tubular necrosis infiltrated with mononuclear cells infiltration as reported in previous studies (Kandemir et al., 2020; Bedir et al., 2021; Sengul et al., 2021). These histological changes occurred by ACR are consistent with the oxidative stress and the subsequently increased renal biochemical parameters as it caused severe kidney damage. The supplementation of TQ had a significant improvement in the renal histological structure as it counteracted the ACR induced toxic effect.

5. CONCLUSIONS

ACR caused oxidative damage to the kidney, which was generated by increases in the renal function biomarker in serum as well as disruptions in the oxidant/antioxidant system. However, co-treatment with TQ mitigated the negative effects of ACR, most likely by increasing cellular antioxidant defenses and decreasing apoptotic tissue damage. Remarkably, the use of TQ is strong antioxidants that have been shown to reduce the effect of numerous known nephrotoxic substances.

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