Ameliorative effect of coenzyme Q10 against deltamethrin-induced renal toxicity in broiler chickens

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ABSTRACT

The ameliorating effect of Coenzyme Q10 (CoQ10) supplementation was evaluated on broiler chickens intoxicated with deltamethrin (DM) through serum biochemical analysis, antioxidant capacity, and pathological examination. Cobb broiler chicks (60) aged 1 day were allocated into 4 experimental equal groups. For each group, three replicates of 5 chicks were used. The first group (control) received only the basal diet, the second group received CoQ10 (40 mg/kg diet), the third group received DM (300 mg/kg diet), and the fourth group received both DM (300 mg/kg diet) and CoQ10 (40 mg/kg diet). The experimental period was 35 days has been given to the last 3 groups. DM intoxication was associated with significant increases in creatinine, urea, malondialdehyde (MDA), and a drop in levels of reduced glutathione (GSH) and superoxide dismutase (SOD). In addition, DM increased blood cholesterol, triacylglycerols, and low-density lipoprotein (LDL), while lowered high-density lipoprotein (HDL). Caspase-3 and B cell lymphoma 2 (BCl2) were substantially unregulated by DM in the kidney tissues. The microscopic examination of the kidneys revealed congestion of the renal blood vessels with necrosis of the lining epithelium of the renal tubules. Concurrent supplementation of CoQ10 with DM resulted in a notable improvement in estimated parameters compared to the DM group. Dietary CoQ10 is therefore advised because of its preventive properties against DM-induced renal toxicity in broilers.

1. INTRODUCTION

Pesticides containing organophosphorus have played a crucial role in enhancing agricultural production for many years, however, they will soon be replaced with safer ones (Kumar et al. 2016). Several agricultural countries now prefer pyrethroid pesticides due to their rapid environmental breakdown and low mammalian toxicity, as well as their greatest insecticidal efficacy (Ogaly et al. 2015). As a type-II pyrethroid, DM controls pests in agriculture, cattle, and poultry production as well as human houses (Siwicki et al. 2010). DM was mainly used to protect crops. Birds, living in the same ecosystem are at risk of exposure to DM (Allam et al. 2022; Chandra et al. 2013; Ibrahim et al. 2021). The human body is highly exposed to DM residues via polluted crops, water, and animal feedstocks, as well as from occupational exposure to DM residues in the workplace (Swarnam and Velmurugan 2013). The kidneys, which responsible for excreting metabolic and waste products, are also damaged by DM exposure (Gündüz et al. 2015; Liu et al. 2015). The harmful effects of DM on many organs can be explained by the accumulation of ROS (Kumar et al. 2016). As a result, enhancing the antioxidant system is essential to prevent the harmful effects of oxidative stress caused by DM exposure. The antioxidant system of poultry can be enhanced using many feed additives such as probiotics, phytoecgenic feed additives (Abdel-Latif et al. 2018; Saeed et al. 2020). CoQ10 is a lipophilic vitamin-like quinine derivative containing 10 isoprenyls units (Nepal et al. 2010). The mitochondrial respiratory chain requires it for electron transport and stability (Shukla and Dubey 2018). DNA, cellular proteins, and membrane lipids are protected from free radical damage by CoQ10, especially in organs with high energy demand, such as the heart, liver, and kidney (Gueven et al. 2015). CoQ10 has a protective effect on hepato-renal toxicity (Geng and Guo 2005; Mwaeni et al. 2021). Also, CoQ10 is documented to have a potent free radical scavenging activity that helps to maintain the mitochondrial membrane potential and to decline protein oxidation, and DNA damage; so, it can restore cell function when subjected to oxidative stress (Abdeen et al. 2020).

In broiler chickens, DM caused renal toxicity (Ibrahim et al. 2021), however, there are no data on the preventive
effects of CoQ10. Therefore, this study set out to evaluate the protective impact of CoQ10 on DM-induced renal toxicity in broiler chickens.

2. MATERIAL AND METHODS

2.1. Chemicals

DM (Butox® 50 mg/ml; Intervet Co., France). CoQ10 was kindly supplied as (Coenzyme Q10® 30 mg) from MEPACO, Cairo, Egypt. The commercial kits used for biochemical and antioxidant biomarkers were obtained from Biodiagnostic Co, Egypt.

2.2. Experimental Animals

Sixty Cobb broiler chicks (one day) were obtained from El-Wataniya Poultry Company, Egypt. These chicks were housed under hygienic measures in separate units. The temperature starts at 32°C, they declined to 2°C each week. Feed and water were supplied ad-libitum and continuous lighting was used.

2.3. Experimental design

The chicks were divided into 4 groups, each was subdivided into 3 replicates (5 chicks each). The 1st group (control group) received basal diet only; 2nd group (CoQ10 group) supplemented with CoQ10 40 mg/kg diet (Gopi et al. 2015), 3rd group (DM group) received a DM at 300 mg/kg diet (Ibrahim et al. 2021) and 4th group (DM: 300 mg/kg diet +CoQ10; 40 mg/kg diet) and all treatments performed for 35 days. Ethical Committee (Faculty of Veterinary Medicine, Benha University) approved the design of this experimental study (Approval number: BUFVTM 03-01-22).

2.4. Sampling

2.4.1. Blood sampling

At the end of the study, blood was obtained from all chickens in different groups from wing veins in dry, clean tubes. Blood was left at room temperature in a slope position to clot. Serum was gathered by centrifugation (10 min at 2000 g), transferred to dry, clean vials, and frozen at −20 °C until used for biochemical analyses.

2.4.2. Tissue sampling

Chickens were dissected and the kidneys were collected, washed with physiological saline, and divided into three portions. One part was homogenized within potassium phosphate buffer and centrifuged (20 min at 1600 g at 4°C). The supernatant was stored at -20°C for the determination of oxidative stress markers. Another part was kept in -80°C till analysis of gene expression. The last portion was fixed in a 10% formalin solution for histopathological examination.

2.5. Biochemical analysis

Urea (Cat. No. UR 2110) and creatinine (Cat. No. CR 1250) were determined in the serum by Coulombe and Favreau (1963); Larsen (1972), respectively. Serum total cholesterol (Cat. No. CH 1220), triglycerides (Cat. No. TR 2030), and HDL-C concentrations (Cat. No. CH 1230) were determined according to Burstein et al. (1970); Stein and Myers (1995); Young et al. (1975), respectively and serum LDL-C concentration was calculated (Friedewald et al. 1972).

The oxidative stress markers were assessed in kidney tissues, MDA as an indicator of lipid peroxidation was determined (Ohkawa et al. 1979). The activity of SOD (Nishikimi et al. 1972), and GSH level were assessed (Beutler 1963). All Kits were obtained from Biodiagnostic CO, Giza, Egypt.

2.6. Quantitative real-time PCR (qRT-PCR) and gene expression

Total RNA was extracted from kidney tissue using RNeasy Mini Kit (Qiagen, USA, Cat. No. 74104) and determined for purity at 260/280 nm. Then, cDNA was synthesized using a High Capacity cDNA Reverse Transcription kit (Qiagen, USA, Cat. No. 205311). The primer sequence was: sense (5'-TGGGCCCTTTGAACCTGAAAC-3') and antisense (5'-TCACTGTGCTGGTCATACC-3') for caspase-3 and since (5'-ATCGTCGCTTCTCTGAGTTT-3') and antisense (5'-ATCCCCATCTCCTCGTGTTCTT-3') for Bcl2. Chicken β-actin was used as a housekeeping gene. The β-actin primer used was: sense (5'-CCACCGCAAAATGCTTCAAAAC-3') and antisense (5'-AAGACTGTGCTGACACCTTTC-3'). The cycling condition of SYBR Green real-time PCR was: 94°C for 5 minutes (primary denaturation), 40 cycles at 94°C for 15 seconds (secondary denaturation), 60°C for 30 seconds (annealing), and 72°C for 30 seconds (extension stage). Cycle threshold (CT) values were determined using Stratagene MX3005P software. Data were analyzed using the 2−ΔΔct method.

2.7. Histopathology

Small tissue specimens were collected from the sacrificed chickens and immediately fixed at least 24 hrs in 10% formalin solution. The fixed tissues were washed, dehydrated in ascending series of ethanol, then tissue paraffin sections sliced into sections (4 µm thick). All slices cleared in xylene and stained with hematoxylin and eosin (H&E) then examined using a Leica DM3000 microscope.

2.8. Statistical analysis

All analysis was conducted with the program SPSS 25. (SPSS Inc., Chicago, USA). Data were mean ± SE, a one-way variance analysis (ANOVA) followed by Duncan’s post-hoc test was used to compare group means. P values of less than 0.05 were considered significant.

3. RESULTS

3.1. DM and/or CoQ10 effect on kidney serum biomarkers

The DM group showed an observable increase in creatinine and urea concentrations (Figure 1 A, B) compared to the control. In addition, DM and CoQ10 showed a remarkable reduction in these parameters compared with the DM group.

3.2. Effect of DM and/or CoQ10 on lipid profile

DM induced significantly higher levels of serum cholesterol, triglycerides, and LDL-C (Figure 1 C, D, E) as well as a markedly lower level of HDL-C (Figure 1 F) compared to the control. However, concurrent supplementation of CoQ10 with DM resulted in an enhancement in cholesterol, triacylglycerols, LDL and HDL serum concentrations compared to the DM group.

3.3. DM and/or CoQ10 effect on renal oxidative stress markers

As illustrated in Figure (2), DM toxicity was accompanied with a marked increases the MDA level (Figure 2A), decreased SOD activity (Figure 2B), and reduced GSH
level (Figure 2C) in kidney tissues when compared to control. However, the DM+CoQ10 group showed a substantial amelioration in renal antioxidant status compared to the DM group. Interestingly, SOD activity and GSH level were almost restored to the control values.

3.4. Effect of DM and/or CoQ10 on Caspase-3 and BCl2 gene expression
DM exhibited marked upregulation of Caspase-3 and a significant downregulation in BCl2 in the kidney. However, a diet supplemented with CoQ10 induced dramatic deregulation of Caspase-3 (Figure 2D) and a notable upregulation of BCl2 (Figure 2E) in the kidney. When DM+CoQ10 was compared to the DM group, an observable downregulation of renal Caspase-3 expression and remarkable upregulation of renal BCl2 expression were recorded. Of note, CoQ10 supplementation to DM intoxicated broiler chickens restored renal BCl2 gene expression to control values.

3.5. Histopathological examination
The examined kidneys of the control and CoQ10 groups (Figure 3A, B) showed well-organized renal tubules which consisted of the proximal convoluted tubule (PCT), distal convoluted tubule (DCT), collecting ducts, and renal corpuscles. However, the DM group (Figure 3C) revealed congestion of the renal tubular epithelium with necrosis of the lining epithelium of the renal tubules. In contrast, the DM+CoQ10 group (Figure 3D) showed cloudy swelling of some renal tubules and detachment of some epithelium lining few renal tubules and swelling of the DCT.

Figure 1: Effect of DM (300 mg/kg diet) and/or CoQ10 (40 mg/kg diet) on urea (A), creatinine (B), cholesterol (C), triglycerides (D), HDL-C (E), and LDL-C (F) in broiler chickens.
Figure 2: Effect of DM (300 mg/kg diet) and/or CoQ10 (40 mg/kg diet) on renal oxidative stress markers; MDA level (A), SOD activity (B) GSH level (C) and on renal gene expression of Caspase-3 (D) and BCl2 (E) in broiler chickens.
4. DISCUSSION
In the present study DM toxicity was associated with severe alterations in kidney function which confirmed by biochemical analysis and histopathological examination which consistent with the previously recorded nephrotoxicity in mice (Tewari et al. 2018) and humans (Valcke et al. 2017). The metabolic product of DM is mainly excreted through the urinary tract (Lin et al. 2011) that could be accused of the recorded nephrotoxic changes in the DM group.
DM intoxicated broiler chickens exhibited an elevation in serum cholesterol and triglycerides. These results concur, well with Han et al. (2020); Tewari et al. (2018), which may be explained by the fact that DM affects cellular permeability and lipid metabolism (El-Sayed and Saad 2008).

The overproduction of free radicals was the most prominent mechanism of DM toxicity (Narra et al. 2017; Hattab et al. 2015). This study indicated that DM induced a remarkable state of oxidative stress in the kidney. During oxidative phosphorylation, mitochondria produce a considerable amount of ROS (Lv et al. 2020) which were able to induce massive cellular oxidative damage if they are not scavenged by endogenous antioxidants (Yang et al. 2016). Chronic exposure to pesticides resulted in excessive lipid peroxidation in various organs and exhaustion of cellular antioxidant defensive mechanisms (Abdel-Daim et al. 2020; Abdou et al. 2020; Soliman et al. 2020 a, b; Zhang et al. 2017), as seen in the DM group.
Also, In the present investigation, DM exposure significantly altered the gene expression of apoptotic and anti-apoptotic markers, which provides additional support for apoptosis as a possible mechanism by which DM may induce kidney damage. The expression of caspase-3 as apoptotic protein was increased in our study. Moreover, Maalej et al. (2017) reported that DM administration induced apoptosis through up-regulation of p53 and Cyclo-

Figure 3: Photomicrographs of the kidneys chickens treated with DM (300 mg/kg diet) and/or CoQ10 (40 mg/kg diet). A: control, B; CoQ10, C: DM and D; DM+ CoQ10 groups. H& E stain.
oxygenase 2 in the kidney tissues. In the current study, the downregulation of BCl2 expression, an anti-apoptotic protein, confirmed their results since it is inversely related to p53.

The most remarkable result to emerge from the data is that feeding a diet supplemented with CoQ10 to DM intoxicated broiler chickens exhibited a significant amelioration of the damaging effects on kidney tissues induced by DM. Serum biochemical and histopathological findings demonstrated the potential effects of CoQ10 in renal protection. This finding was in complete agreement with Albadrany and Naser (2020). A possible theory for CoQ10’s protection from DM toxicity may be the impact of its antioxidant action. The ability of CoQ10 to maintain an antioxidant/pro-oxidant balance (Sohal and Forster 2007; Ghule et al. 2009) counteracts the production of ROS during DM metabolism and excretion.

The current study showed that CoQ10 supplemented diet remarkably attenuated the alteration in lipid profile resulting from DM exposure. This fits well with the previous study of Albadrany and Naser (2020). The decline in serum cholesterol level reported could be due to HMGCoA reductase inhibition, the key enzyme in cholesterol synthesis (Honda et al. 2009). Consequently, LDL production decreased. There may be another possible explanation, the antioxidant behavior of CoQ10 protects LDL oxidation by harmful ROS (Singh et al. 2007). In the same line, CoQ10 attenuates tissue damage, which prevents disturbances in fatty acid metabolism. This could explain the decrease in serum triacylglycerols.

As expected, concurrent supplementation of CoQ10 to DM intoxicated broiler chickens significantly improved both enzymatic (SOD) and non-enzymatic (GSH) antioxidants and diminished MDA production in kidney tissues. Our results were confirmed by previous studies (Gopi et al. 2014; Huang et al. 2011; Maalej et al. 2017). Many possible explanations might explain our findings such as CoQ10 suppresses ROS overproduction, ROS quenching, and endogenous antioxidant maintenance (Ratliff et al. 2016).

Also, the results of this experiment reveal CoQ10 to be anti-apoptotic. The current study demonstrated that co-administration of CoQ10 with DM was accompanied with downregulation in caspase 3 and up-regulation in BCL2 expression of renal tissues. These results were in agreement with Abdeen et al. (2020). Kidneys observed a downregulation of caspase-3. The anti-apoptotic activity of CoQ10 could be attributed to its capability to regulate the perturbation electrochemical gradients on the mitochondrial level (Formigli et al. 2003). Consequently, the presence of congestion of the renal tubular epithelium with necrosis of the lining epithelium of the renal tubules in DM treated group. In contrast, the DM+CoQ10 group showed cloudy swelling of some renal tubules and detachment of some epithelium lining few renal tubules. It is strongly reported that the inflammatory response is associated with oxidative stress; herein, the inflammatory cell infiltrations were seen in the kidney tissue after DLM exposure (Allam et al. 2022; Ibrahim et al. 2021). All of the detected pathological lesions improved with CoQ10 treatment.

CoQ10 acts as a potent antioxidant free radical scavenger, thus limiting damage associated with oxidative stress (Abdeen et al. 2020). CoQ10 in the diet improved reproductive performance (Rafieian-Naeini et al. 2021).

Conclusions
DM induced disturbance in renal function, lipid peroxidation, glutathione function, and antioxidant enzymes. CoQ10 acts as a potent antioxidant free radical scavenger, thus limiting damage associated with oxidative stress and so antagonize this harmful effect of DM on the kidney of chickens.

6. REFERENCES


