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RESEARCH ARTICLE

Testicular Injury of Acrylamide in Rats and Potential Protection of Coenzyme Q10 and Rosuvastatin

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A B S T R A C T

Acrylamide (ACR) is created when foods high in carbohydrates are heated up and is more harmful because it produces ROS inside cells. Our research aimed to investigate the potential of coenzyme Q10 (CoQ10) and rosuvastatin (ROSV) in mitigating the adverse effects of testicular toxicity induced by ACR in rats. A total of 49 male rats were distributed among 07 groups. Group 1 served as the control and was administered normal saline orally, while group 2 received oral CoQ10 at a dosage of 10 mg/kg BW; group 3, was administered ROSV (10 mg/kg/BW, orally); group 4, received ACR orally (20 mg/kg/BW); group 5 received ACR and CoQ10; group 6 administered ACR and ROSV; and group 7, the rats received ACR, CoQ10, and ROSV daily for 30 days. Acrylamide lowered serum testosterone, FSH, and LH levels, and marked elevations in testicular MDA levels were observed alongside notable decreases in GSH, CAT and SOD. It caused a significant reduction in the diameter of seminiferous tubules, numbers of spermatocytes, spermatogonia, Sertoli cells, germinal epithelium height, and a dramatic increase in lumen diameter. Also, the testicular histopathology was disturbed by ACR, and the testicular caspase-3 expression was considerably elevated in the ACR-treated group. In rats exposed to ACR intoxication, CoQ10 and ROSV raised levels of antioxidant biomarkers, lowered MDA and mitigated alterations in serum hormonal parameters. In summary, our results suggest that CoQ10 and ROSV exhibit considerable protective effects against ACR-induced testicular toxicity, attributed to their anti-oxidative properties.

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INTRODUCTION

Acrylamide (ACR) is a commonly used chemical compound in a variety of industries. It is produced when carbohydrate-rich foods are processed at high temperatures (>120°C) which leads to serious human health problems. ACR is present in the majority of bakery products, such as toast, bread, potatoes, biscuits, chips, coffee, and fries (Sarion *et al*., 2021). Furthermore, potatoes and cereal grains were included in pet foods manufactured at high temperatures (Kandeil *et al*., 2019). ACR is a highly toxic chemical that is widely used in paper, dyes, and plastic manufacturing (Radad *et al*., 2020). Infertility can be caused by reactive oxygen species (ROS), which damage spermatozoa, testicular function, and Leydig cells. Male infertility appears to be distinguished by testicular oxidative stress, which is induced by sperm production deficiency and hormonal changes (Amanpour *et al*., 2020). Free radicals are eliminated by antioxidants, and cells are protected from potentially dangerous oxidative reactions (Elsayed *et al*., 2022). Also serious human health problems were associated with ACR exposure such as

carcinogenicity (Sharafi *et al*., 2024) and neurotoxicity (Aboubakr *et al*., 2023b).

One of the main factors in the process of tissue destruction is oxidative stress. It happen when there is an imbalance between the body's capacity to counteract or repair the damaging effects of reactive oxygen species (ROS) and the synthesis of these molecules (Aboubakr *et al*., 2023a). So it is important to investigate potential protective agents against its toxicity.

Coenzyme Q10 (CoQ10) is a vital molecule that resembles a fat-soluble vitamin. It is naturally found in all cellular membranes and mitochondria (Irma *et al*., 2022). CoQ10 is present in nature in foods that are abundant in this molecule, such as fish, meat, broccoli, and cereals. CoQ10 plays a crucial role in the generation of energy within mitochondria by actively participating in both the mitochondrial respiratory chain and the transportation of electrons outside of mitochondria (Shabaan *et al*., 2021). By effectively scavenging free radicals and inhibiting the peroxidation of lipids, CoQ10 exhibits strong antioxidant properties (Hossain *et al*., 2023).

Statins offer a protective effect that goes beyond their primary function of reducing cholesterol levels. Rosuvastatin (ROSV), along with other statins, has demonstrated a diverse range of activities, including anti-inflammatory and antioxidant properties, which can vary among different statins (Mansour *et al*., 2021). ROSV has been effective in reducing oxidative stress associated with renal toxicity (Abdeen *et al*., 2019). Moreover, the antioxidant and anti-apoptotic effects of ROSV were observed in the liver and kidney tissues of rats subjected to fipronil exposure (Abdel-Daim and Abdeen, 2018).

The purpose of this study was to look into the role of CoQ10 and/or ROSV in preventing testicular toxicity caused by ACR in rats by looking at testosterone, FSH, and LH levels, oxidative stress indicators, and morphometrical parameters.

MATERIALS AND METHODS

Chemicals: The ACR used in this study was obtained from Sigma-Aldrich in the USA. CoQ10 (Coenzyme Q 10®, 30 mg) was purchased from MEPACO, Cairo, Egypt, while ROSV (Crestor®, 20 mg) was obtained from AstraZeneca Company, Giza, Egypt.

Experimental design: A total of 49 male Wister Albino rats weighing between 185-200 g were obtained from the Egyptian Organization for Biological Products and Vaccines. The rats were acclimatized for 7 days at a temperature of (25±2°C), during which they were provided with unrestricted access to water and commercial pellets. The rats were randomly assigned to seven main groups, each containing seven rats. Group 1 was designated as the control group and received an isotonic saline solution. Group (2): treated with CoQ10 (10 mg/kg BW) (Saha *et al*., 2019); Group (3) treated with 10 mg ROSV/kg BW (Aledani *et al*., 2022); Group (4) intoxicated with ACR (20 mg/kg BW) (Aboubakr *et al*., 2019); Group (5): ACR + CoQ10; group (6) treated with ACR + ROSV and group (7) treated with ACR+ CoQ10+ ROSV. Each treatment, including saline, CoQ10, ROSV, and their combinations, was orally administered once daily for 30 days.

Sampling (Blood & tissues): Following the end of the experiment, the rats were anesthetized with isoflurane 24 hours later. Blood samples were obtained from the retro-orbital plexus, and the serum was centrifuged at 1200 g for 15 minutes before being stored at -20°C for subsequent hormone analysis. The testes were removed and rinsed with saline. Tissues weighing one (1) gram were homogenized in a phosphate buffer with a pH of 7.4. The homogenized tissues underwent centrifugation at 1200 x g for 20 minutes at 4°C. The resulting supernatants were stored at -20°C until utilized for assessing oxidative stress markers in the testicular tissues. A portion of the testicular tissue was promptly fixed in formalin to facilitate histopathological and immunohistochemical (IHC) evaluations.

Hormone level analysis: Serum testosterone, FSH, and LH hormone levels were measured using specific ELISA kits, following the manufacturer's protocol. The ELISA kits were obtained from MyBioSource Co, San Diego, USA.

Oxidative stress: Testicular tissue levels of MDA, SOD, CAT, and GSH were assessed using diagnostic kits sourced from Biodiagnostic Co, Egypt.

Morphometry, lesion scoring, histopathology, and immunohistochemistry (IHC): Morphometric analysis of testes of numbers of spermatocytes, spermatogonia, Sertoli cells, the germinal epithelium (height), seminiferous tubules, and lumen diameters was measured by using Image J software. Scoring of the testicular lesion based on the number and severity of the lesion was illustrated in Table 1.

The testes were embedded in paraffin wax and fixed with 10% formaldehyde. A microtome instrument was employed to slice sections of paraffin blocks, with a thickness of 5 micrometers. Before staining with H&E, the tissue sections were mounted on glass slides and underwent deparaffinization, which involved the removal of the paraffin wax from the samples. Caspase 3 immunostaining was used according to Porter and Jänicke (1999).

Statistical analysis: The results obtained were presented as mean ± SD. Statistical analyses were performed using GraphPad Prism 9 software from San Diego, CA, USA, incorporating one-way ANOVA and Tukey's post hoc test for multiple comparisons. A significance level of $P \leq 0.05$ was applied.

RESULTS

Effect on serum hormone levels: ACR-induced testicular toxicity was demonstrated by lower testosterone, FSH, and LH levels than the control. When CoQ10 and ROSV were combined and applied to rats intoxicated by ACR, there was a notable increase in these hormones, which was markedly lower than in the control group. The findings of the study suggest that the combined administration of CoQ10 and ROSV showed better protection against ACR-induced reproductive damage when compared to the use of either supplement alone, as shown in Fig. 1.

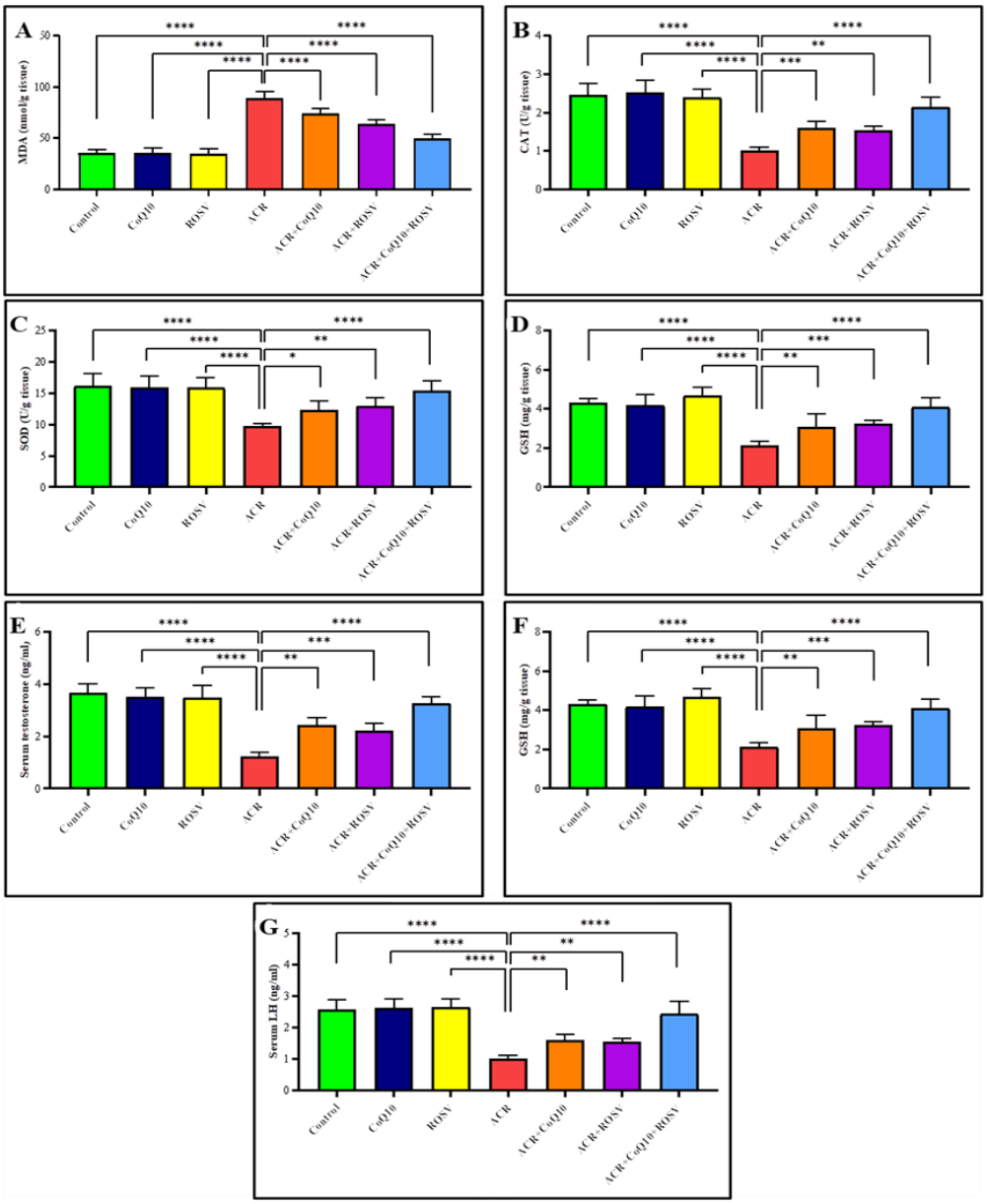

Fig. 1: Effect of CoQ10, ROSV, and/or ACR on serum testosterone, FSH, LH, and antioxidant parameters testicular tissues. Data are expressed as the mean±SD (n=7).

Table 1: Scoring of testicular lesion based on the number and severity of the lesion.

Parameters	Control	CoQ10	ROSV	ACR	ACR+CoQ10	ACR+ROSV	ACR+ CoQ10+ROSV
Inflammatory cells	-	-	-	++++	++	++	+
Odema	-	-	-	+++	++	++	+
Congestion	-	-		++++	+	+	-
Degeneration	-	-	-	++++	++	++	+
Desquamation	-	-	-	++++	+	+	-
Regeneration	-	-	-	-	++	++	+++

- (Negative), + (Mild), ++ (Moderate), +++ (Highly positive) and ++++ (Sever positive).

Effect on oxidative damage parameters: Significant increases in MDA levels with a dramatic decrease in GSH, SOD and CAT levels in ACR tissues of the testis. The results indicated that the administration of either CoQ10 or ROSV alone resulted in a notable decrease in the adverse effects of ACR on MDA, SOD, CAT and GSH levels in the testes, although the values were still considerably different from those of the control group. In addition, group (ACR+CoQ10+ROSV) had a statistically significant higher level of ACR-induced oxidative damage in the testes compared to group (ACR+CoQ10) and (ACR+ROSV) as shown in Fig. 1.

Morphometry, lesion scoring, and histopathology: ACR caused a significant reduction in the diameter of seminiferous tubules, numbers of spermatocytes, spermatogonia, Sertoli cells, germinal epithelium height, and a dramatic increase in lumen diameter (Fig. 2). Also, the administration of CoQ10 and ROSV along with ACR notably restored these changes. The morphometrical markers in the control, CoQ10 and ROSV-treated groups were nearly identical.

Scoring of the testicular lesion (Inflammatory cells, edema, congestion, degeneration, desquamation, and regeneration) based on the number and severity of the lesion was illustrated in Table 1.

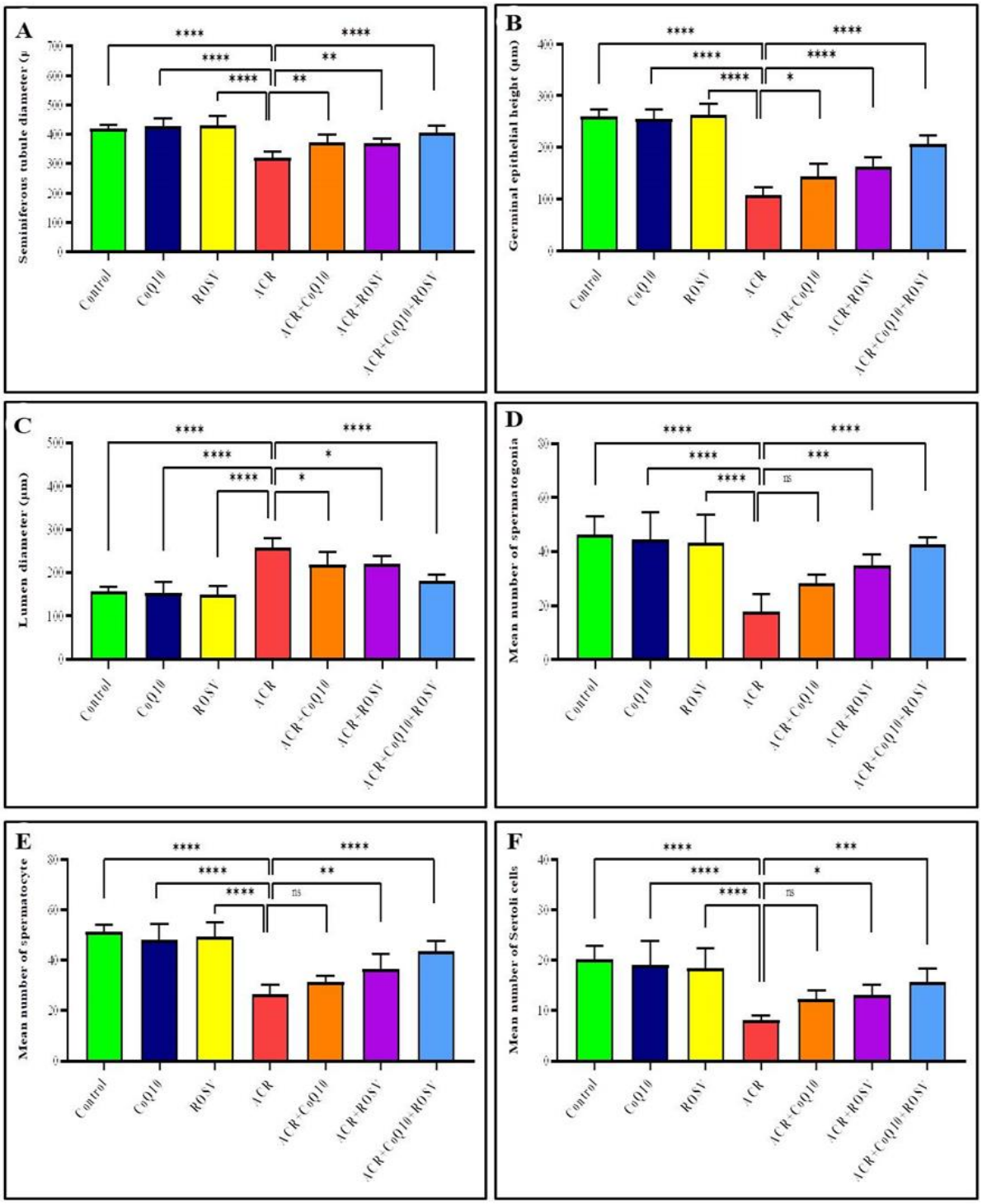

Fig. 2: Effect of CoQ10 and/or ROSV on morphometrical parameters of testes tissue in rats treated with ACR. Data are expressed as the mean±SD (n=7).

The histological structure of seminiferous tubules, including the arrangement of spermatogenic cells and interstitial tissue, appeared normal in the sections from the control, CoQ10, and ROSV groups (Fig. 3A-C). Testes from ACR treated group demonstrated marked degenerative changes and disrupted testicular architecture including vacuolation and degeneration of spermatogonia and spermatocytes (Fig. 3D), exfoliation of multinucleated giant cells into the lumen and among spermatogenetic cells (Fig. 3E) as well as widening of interstitium with an accumulation of edematous fluids (Fig. 3F). However, testicular sections from the ACR+CoQ10 and ACR+ROSV treated groups exhibited amelioration of degenerative alterations compared to ACR-exposed rats but, with slight interstitial edema (Fig. 4A) and congested interstitial blood vessels (Fig. 4B) were seen respectively. Meanwhile, the group treated with ACR+CoQ10+ROSV showed a recovery of the typical structure of the testes, which was observed through the presence of normal interstitial tissue and spermatogenic cells (Fig. 4C).

Immunohistochemistry: The immunohistochemical expression of caspase3 in the ACR group revealed significantly stronger immunoreaction in most of the spermatogenic cells (Fig. 5D) compared to the weak

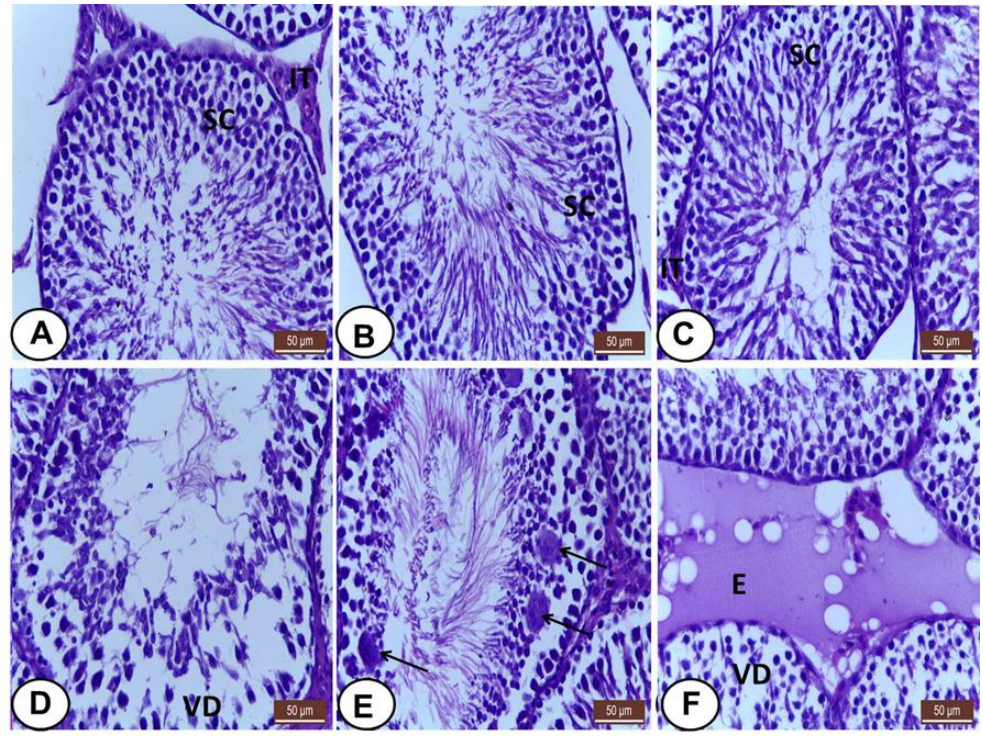

Fig. 3: Histopathological sections of testes from control, CoQ10, ROSV, and ACR groups. A B and C; control, CoQ10, and ROSV groups showed a normal arrangement of spermatogenic cells (SC) as well as normal interstitial tissue (IT). D-F; ACR treated rats showed several histological changes; vacuolation and degeneration of spermatogonia and spermatocytes (VD), exfoliation of multinucleated giant cells into the lumen and among spermatogenetic cells (arrows) as well as widening of interstitium with an accumulation of edematous fluids (E). H&E stain, scale bars=50µm.

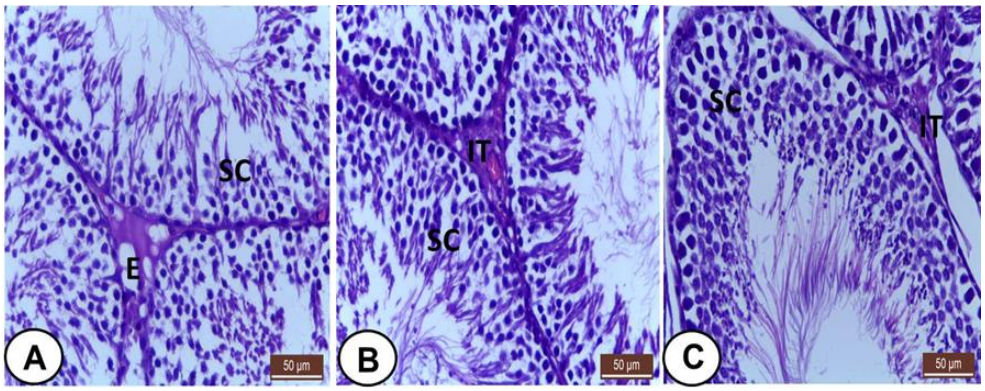

Fig. 4: A, B and C showed histological sections of testes from ACR+ CoQ10, ACR+ ROSV and ACR+ CoQ10+ ROSV groups respectively. A and B; show an improvement of the degenerative changes compared to ACR-exposed rats but, with slight interstitial edema (E) and congested interstitial blood vessels (IT). C; showing restoration of the normal testicular architecture of normal spermatogenic cells (SC) and interstitial tissue (IT). H&E stain, scale bars=50µm.

caspase 3 immunoreactivity observed in the testicular sections from the control, CoQ10, and ROSV groups, a significant difference was observed (Fig. 5A-C). However, in the ACR+CoQ10 and ACR+ROSV groups, the caspase3 immunoreactions were observed to be moderate (Fig. 5E, F) compared to the ACR group. Weak caspase3 immunoreaction was seen in spermatogenic cells of the ACR+CoQ10+ROSV group (Fig. 5G) which was nearly similar to that of the control group.

DISCUSSION

Acrylamide is known to cause significant damage to the seminiferous tubules, indicating that the testis is a vulnerable target of its action. Moreover, ACR is highly toxic to multiple organs and has been associated with increased oxidative stress and depletion of antioxidants (Lebda *et al*., 2014). Oxidative stress is the primary mechanism underlying ACR toxicity. ACR and its metabolite, glycidamide, form covalent adducts with DNA, which causes mutations, DNA damage, and carcinogenicity (Farag *et al*., 2021). Also, ACR and glycidamide can form adducts with the thiol groups of the majority of biomolecules, depleting the intracellular thiols that activate the LPO (Baba and Bhatnagar, 2018).

Sex hormones are among the biological markers that have been reported to assess toxicities and reproductive functions (Sahreen *et al*., 2013). The administration of ACR to rats led to testicular toxicity, as indicated by alterations in reproductive function such as the suppression of spermatogenesis, testicular androgen production and gonadotropic secretion. Acrylamide reduces the levels of testosterone, LH and FSH in the blood. This observation is in line with earlier studies that reported a reduction in the levels of male sex hormones (Erdemli *et al*., 2019). FSH, LH, and testosterone regulate and maintain testicular function. In males, FSH regulates and promotes spermatogenesis. Naturally, the primary hormone for sperm production in the testis is testosterone (Regueira *et al*., 2015). It is known that disturbance of testicular steroidogenesis, activation of hepatic androgen metabolism into metabolic products with low androgen receptor binding activity, or direct damage caused by ACR to Leydig cells may be the causes of the harmful effects of ACR on serum testosterone (Yassa *et al*., 2014).

Testicular oxidative stress can harm Leydig cells, which can change testosterone production and is a common aspect of infertility (de Souza Santos *et al*., 2004). CoQ10 and/or ROSV supplementation restored serum levels of testosterone, LH, FSH, and sperm

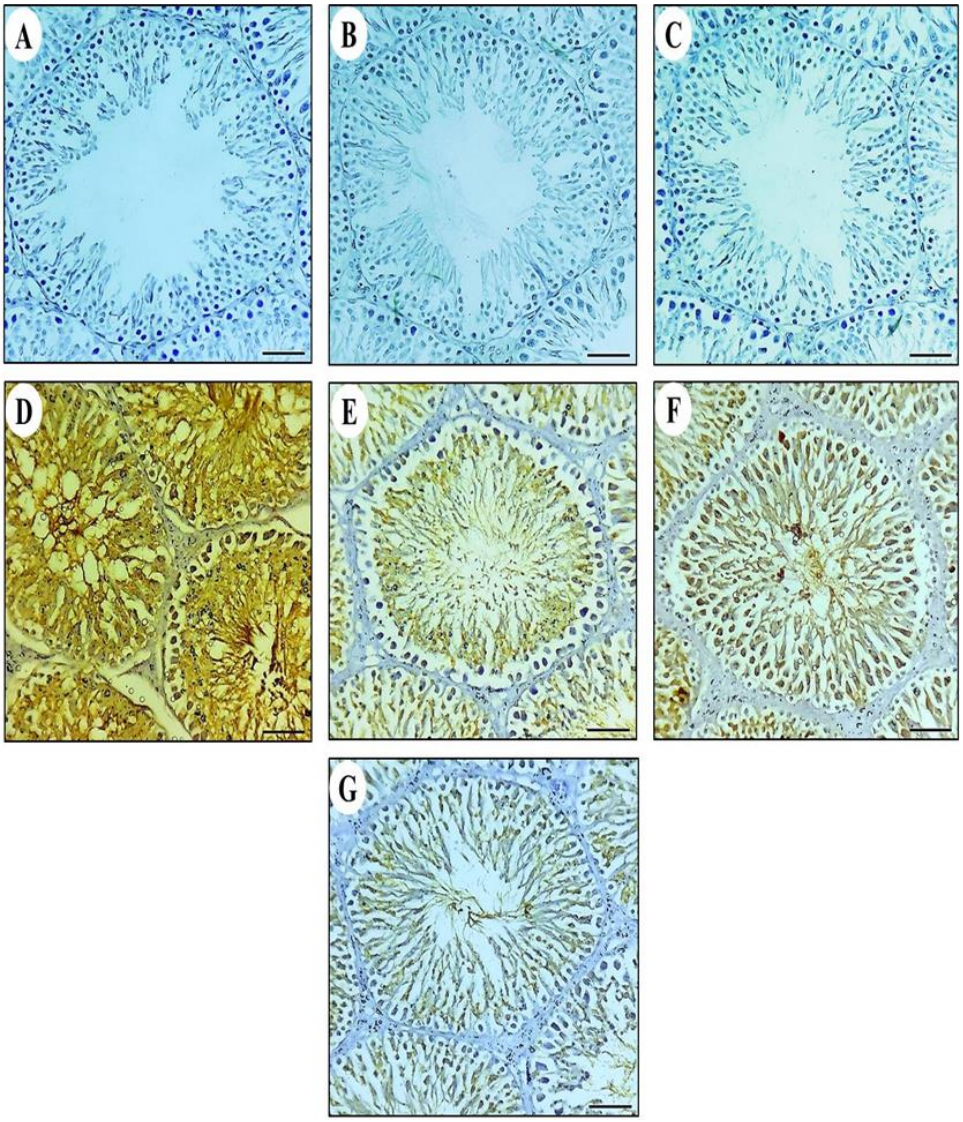

Fig. 5: Immunohistochemical staining of caspase 3 in testicular sections from all examined groups. A, B and C; Control, CoQ10, and ROSV groups showed weak caspase 3 staining in the spermatogenic cells. D; ACR group revealed strong caspase 3 staining in the spermatogenic cells. E-F; ACR+ CoQ10 and ACR+ ROSV groups respectively showed moderate immunoreaction in comparison to the ACR group. G; ACR+ CoQ10+ ROSV group revealed weak caspase3 immunoreaction which was nearly similar to that of the control group.

parameters to near the control levels in ACR-intoxicated rats. These findings were consistent with previous research that found that CoQ10 treatment significantly mitigated the decrease in serum testosterone levels induced by arsenic (Fouad *et al*., 2011). CoQ10 supplementation, along with lead acetate, led to an improvement in testosterone, LH, and FSH levels, as documented by El-Khadragy *et al*. (2020). Also, treatment with ROSV resulted in decreased testosterone and LH hormone levels, along with histopathological and morphometric changes in testicular tissues in rats (Aledani *et al*., 2022). Simvastatin elevated serum testosterone levels in rats subjected to testicular toxicity induced by cadmium (Fouad *et al*., 2013).

The current findings suggest that oxidative stress is a result of ACR administration by elevating MDA and lowering GSH, SOD, and CAT levels, which may be one of the constituents of sperm morphological abnormalities. Spermatozoa membranes' lipid matrix is damaged when lipid peroxides are liberated, which reduces sperm motility, sperm count, and spermatogenesis (Gürler *et al*., 2016).

Furthermore, to the body's natural antioxidant defense mechanisms, supplemental antioxidant agents are crucial in preventing tissue damage by lowering ROS levels (Conti *et al*., 2016). One of the most significant byproducts of lipid peroxidation, elevated MDA levels alter ion exchange across cell membranes, causing cross-linking of substances in the membrane and having detrimental effects like altering enzymatic activity and ion permeability elevated lipid peroxidation results from a specific level of GSH reduction (Yousef *et al*., 2006). As a result, a decrease in GSH levels within a cell may play an important role in ACR-induced genotoxicity (Lamy *et al*., 2008). Coenzyme Q10, a naturally occurring hydrophobic compound, plays essential roles in the mitochondrial respiratory chain and functions as a potent antioxidant (Fouad *et al*., 2011). Moreover, CoQ10 protects the testis against various types of damage (Najafi *et al*., 2019). The administration of CoQ10 has been shown to effectively mitigate the reduction of antioxidant defense mechanisms (such as SOD and GSH) and suppress lipid peroxidation in testicular tissue caused by arsenic exposure (Fouad *et al*., 2011). The primary factors contributing to the protective effects of CoQ10 on the testicles are its antioxidant and anti-inflammatory properties (Fouad *et al*., 2011). When CoQ10 is administered concurrently with doxorubicin, it effectively restores the levels of MDA, GSH concentration, and CAT activity in the testicles, surpassing the restorative effects

observed in groups treated solely with doxorubicin (El-Sheikh *et al*., 2014). Additionally, CoQ10 treatment has shown positive outcomes in countering testicular toxicity induced by lead acetate (El-Khadragy *et al*., 2020). A significant reduction in MDA levels in mice co-treated with atorvastatin, as compared to mice receiving doxorubicin alone was reported (Ramanjaneyulu *et al*., 2013). Also, the administration of atorvastatin along with doxorubicin showed a significant increase in the GSH level in the testes. Compared to the group of rats exposed to cadmium without simvastatin treatment, the rats treated with simvastatin exhibited notably elevated levels of testicular GSH and catalase activity, along with significantly reduced levels of MDA in testicular homogenates (Fouad *et al*., 2013). Atorvastatin ameliorated the increased level of testicular MDA and it increased the depleted level of testicular glutathione reductase induced by high-fat diet-induced low fertility (Esmail *et al*., 2020).

This study's histopathological findings confirmed the biochemical ones. Compared to the group of rats treated with ACR alone, the rats that received CoQ10 or ROSV showed a significant improvement in testicular degeneration and inflammation caused by ACR treatment. Co-treatment of ACR groups with CoQ10 and ROSV improved testicular tissue significantly. Following ACR intoxication, there was a pro-oxidant/antioxidant imbalance in the testes which was accompanied by histopathological changes represented by degenerative changes and disrupted testicular architecture including vacuolation and degeneration of spermatogonia and spermatocytes, exfoliation of multinucleated giant cells into the lumen and among spermatogenetic cells, as well as widening of interstitium with an accumulation of edematous fluid. Farag *et al*. (2021) reported that ACR induced severe pathological changes. Exposure to ACR led to various negative changes in the testicles, such as degeneration of spermatogenic cells within the seminiferous tubules and interstitial edema that was in harmony with Lebda *et al*. (2014) as well as exfoliation of multinucleated giant cells into the lumen and among spermatogenetic cells which similar to the finding of Kaçar *et al*. (2018). Both CoQ10 and ROSV administration can induce partial protection against ACR testicular toxicity and exhibit improvement of histo-architecture of the seminiferous tubules. CoQ10 ameliorated the histopathological changes induced by the magnetic field (Ramadan *et al*., 2002), arsenic (Fouad *et al*., 2011), doxorubicin (El-Sheikh *et al*., 2014), lead acetate (El-Khadragy *et al*., 2020), and chlorpromazine (Oyovwi *et al*., 2021) were reversed by CoQ10, which may attribute to the free radicals scavenging activity of CoQ10. Furthermore, the administration of atorvastatin alongside doxorubicin resulted in a significant reduction of doxorubicin-induced testicular abnormalities, as evidenced by the nearly normal histological structure observed in the testicular section (Ramanjaneyulu *et al*., 2013). Treatment with simvastatin effectively mitigated the damage caused by cadmium to the testicular tissue, promoting the preservation of active spermatogenesis in the majority of seminiferous tubules (Fouad *et al*., 2013). However, when a combination of reactive oxygen species scavengers (ROSV) and Coenzyme Q10 was used, superior results were observed in countering the testicular toxicity induced by ACR.

The present study demonstrated an increase in Caspase-3 expression in the testes of the ACR-treated group compared to the control group. The group treated with ACR demonstrated notable expression of Caspase-3, which was consistent with the findings reported by Yang *et al*. (2005) in rats and Zhang *et al*. (2009) in mice. The findings indicate that ACR leads to testicular apoptosis. However, supplementation with Coenzyme Q10 and/or ROSV showed significant improvements in the structural changes observed in the testes due to ACR treatment. In rats treated with CoQ10, there were significant reductions in the overexpression of caspase-3 in the testicular tissue induced by arsenic, compared to the group exposed to arsenic without CoQ10 treatment (Fouad *et al*., 2011). Simultaneous administration of Coenzyme Q10 and doxorubicin resulted in a significant reduction of caspase-3 expression in the majority of spermatogenic cells, while minimal immune-reactivity was observed in the interstitial cells of Leydig (El-Sheikh *et al*., 2014). Similarly, treatment with simvastatin significantly reduced the expression of caspase-3 in the cells of seminiferous tubules induced by cadmium, compared to the group exposed to cadmium without simvastatin treatment (Fouad *et al*., 2013). The levels of caspase-3 induced by high glucose levels were significantly reduced by rosuvastatin, potentially due to the anti-oxidative properties of the medication (Heeba and Hamza, 2015). This finding aligns with a previous study that demonstrated the ability of simvastatin pre-treatment to significantly decrease caspase-3 activity in the testes of rats experiencing testicular injury caused by ischemia-reperfusion (Tu *et al*., 2011). The anti-apoptotic effect observed with simvastatin treatment can be attributed to its antioxidant action (Fouad *et al*., 2013).

Conclusions: The study found that apoptosis and oxidative stress contributed significantly to testicular damage following ACR. Taking COQ10 and ROSV simultaneously can prevent testicular damage caused by ACR.

Ethics approval: The ethical treatment of the rats followed approved guidelines for the use of laboratory animals and complied with the regulations of the Research Ethical Committee of the Faculty of Veterinary Medicine at Cairo University, Egypt (Vet CU 08072023721).

Authors' contribution: Elsayed A, Aboubakr M, Abdelhiee EY (methodology). Soliman A; Elfadadny A, Farag A, designed the experimental protocol. El-Shafey A, Hassan FW, Zakaria M, Abdelmageed N; original draft preparation, review and editing. All authors read, revised, and approved the final manuscript.

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