The Protective Effects of Vit E and CoQ10 Against Acetamiprid Induced Reproductive Toxicity in Adult Male Sprague Dawley Rats

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KEYWORDS
Acetamiprid,
Coenzyme Q10,
Vit E,
Caspase-3,
TNFα.

ABSTRACT

Acetamiprid (ACE) is one of the most worldwide used neonicotinoids. The current study aimed at investigating the protective effects of vitamin E (Vit E) (100 IU/kg/day) and/or Coenzyme Q10 (CoQ10) (75 mg/kg/day) against (ACE) (25 mg/kg/day) induced male reproductive toxicity. Eight groups of adult male Sprague Dawley rats (n=6) were used as the following: Control, oil, (Vit E), (CoQ10), (ACE), (ACE + Vit E), (ACE+CoQ10) and (ACE+ Vit E+CoQ10). Rats received treatments by oral gavage for 60 days. The administration of (ACE) showed significantly decreased of the following: (body weights, testicular weights, serum testosterone, serum luteinizing hormone, testicular antioxidant reduced glutathione, testicular superoxide dismutase activity, sperms count and percentage of sperms motility); in line with significantly increased of the following: (percentage of dead sperms, percentage of abnormal sperms and testicular malondialdehyde). Histologically; (ACE) group showed marked degenerative changes in the seminiferous tubules with increased immune histochemical expression of testicular (Caspase-3 and Tumor necrotic factor alpha). Separate administration of either (Vit E) or (CoQ10) with (ACE) showed improvement of ACE induced histological, oxidative, inflammatory, apoptotic, semen toxic effects, but failed to improve serum testosterone, body and testicular weights. Moreover, combined protective effects of (Vit E) and (CoQ10) significantly overcame the separate use of both agents in improving body and testicular weights, sperm motility, vitality, testosterone, luteinizing hormone and Caspase-3 expression. Finally, the administration of either (Vit E) or (CoQ10) showed to have protective effects against (ACE) induced male reproductive toxicity, although the combined use of both agents was more effective.

Introduction

Neonicotinoids are new class of insecticides that act as selective nicotinic acetylcholine receptor agonist mainly in the central nervous system (Zuščíková et al., 2023). Recently, neonicotinoids are widely used as less toxic alternative that replacing organophosphate (Phogat et al., 2022). Acetamiprid (ACE), (N-((6-chloropyridin-3-yl) methyl) -N0 –cyano –N -methyl-ethanimidamide), is one of the chloropyridinyl neonicotinoid (Houchat et al., 2020). Occupational exposure to ACE occurs via skin absorption and inhalation routes during fumigation. Environmental exposure to ACE occurs mainly via ingestion of contaminated food and water (Marın et al., 2004). The ACE residues in water and soil are resistant to hydrolysis and could persist for more than one year (Renaud et al., 2018). In the experimental models, ACE was

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determined by high concentrations and caused significant toxicities in the liver (Shahin, 2018) and kidney (Alhusaini et al. 2019) which represent sites of its metabolism and excretion, respectively. Moreover, it showed to have toxic effects on the nervous system (Dukhnytskyi et al. 2020), and immune system (Marzouki et al., 2017).

In human studies, ACE reported to have acute toxicity manifestations simulating organophosphates but milder (Imamura et al., 2010; Pirasath et al., 2021). Chronic exposure to ACE was associated with decreased sperms count and increased incidence of still birth in farmers (Neghab et al., 2014). The ACE was reported to induce developmental toxicity in case of prenatal exposure (Gu et al., 2013). Male reproductive toxicity induced by ACE was assumed to direct toxic effects on the spermatogenesis as well as endocrine disrupting effect by decease testosterone level (Kong et al., 2017; Arıcan et al., 2020). Various molecular mechanisms have been implicated in ACE toxic effects including oxidative stress, DNA damage and apoptotic effects in both in vitro and in vivo studies (El-Bialy et al., 2020; Gomez et al., 2020; Öztaş et al., 2021). Hence, various antioxidant and natural agents had been investigated to mitigate the ACE toxic effects (Phogat et al., 2022).

Vitamin E (Vit E) (alpha-tocopherol) is a lipid-soluble vitamin which has an antioxidant and membrane stabilizing protective effects in cells of different biological models (Liao et al., 2022). The hydroxyl group in tocopherol reacts with peroxyl free radicals forming tocopheryl radical. The tocopheryl radical returns to the reduced active form of Vit E by reacting with a hydrogen donor e.g., vitamin C (Kong et al., 2017). The Vit E previously reported to ameliorate the hepatorenal toxic effects induced by ACE in rat model (Yi-Wang et al., 2012). In addition, (Zhang et al., 2011; Kong et al., 2017 and Zayman et al., 2022) reported that Vit E had protective effects against the male reproductive toxicity in ACE intoxicated animal models.

Coenzyme Q10 (CoQ10) (ubiquinone or ubidecarenone) is a fat-soluble coenzyme that formed by conjugation of benzoquinone ring with isoprenoid chain (Shukla and Dubey, 2018). The CoQ10 increases the production of ATP and has antioxidant reducing capacity that gets rid of free oxygen radicals. The reduced form CoQ10 (ubiquinol) is a potent antioxidant as it is essential for regeneration of other antioxidants such as ascorbate (Vitamin C) and Vit E (Ognjanović et al., 2010). Hence, there is growing evidence of its potential role in amelioration of various diseases (Shukla and Dubey, 2018; Pallotti et al., 2021) as well as in different organ toxicities as neurotoxicity (Yousef et al., 2019) and hepatorenal toxicity (Oda et al., 2018). CoQ10, alone or in combination with other antioxidants is reported to have a beneficial effect on seminal quality and sperm motility (Allam et al., 2022, Oyovwi et al., 2022). Although the effective CoQ10 dose and best combination with other antioxidants compared to monotherapy are still under investigation (Salvio et al., 2021). The combined effect of CoQ10 and Vit E previously reported to improve male reproductive toxicity induced by single toxic dose of cadmium (Ognjanović et al., 2010). To the best of our knowledge, the protective effect of CoQ10 against ACE induced male reproductive toxicity had not been previously investigated. Hence, the aim of the current work was to investigate as well as compare the potential protective effects of CoQ10 and Vit E against the ACE induced subchronic male reproductive toxicity.

Material and methods:

Chemicals

Acetamiprid (96% TC) in the form of white powder, manufactured by (Jiangsu Feng Shan Co., China), and was obtained from (Delta Co., for chemical industries, Egypt). Vitamin E (Alpha Tocopherol acetate 400 mg Equi. to 400 IU) and Coenzyme Q10 (Coenzyme Q10 Forte 100 mg) were obtained in the form gelatinous capsules from (MEPACO Co., Egypt). All the other chemicals utilized in the study were purchased as analytic grade from (Bio-diagnostic Company, Giza, Egypt).

Animals

A total of 48 adult male Sprague Dawley rats weighing (200–220 g) were obtained from the animal house Faculty of Veterinary medicine, Mansoura University. Animals were housed in plastic cages at an ambient temperature of (20 ± 2^{o} C) and relative humidity (55 ± 5%), as well as light and dark cycle was maintained at 12 h/12 h. The rats were feed freely on commercial pelleted food (Al Wadi Co., Giza, Egypt) and water at the department of pesticides, Faculty of Agriculture, Mansoura University where the experiment was conducted. Rats were handled following the National Institutes of Health (NIH) general guidelines for the care and use of laboratory animals. The present study was authorized by Mansoura university Animal care and use committee code number (AGR.R.23.08.4).

Experimental Design

After one week of acclimatization, rats were divided into 8 groups (each group comprised 6 rats). Repeated doses toxicity classified to: (subacute exposure for two or four weeks, Subchronic exposure for 1-3 months and chronic exposure for more than 3 months up to one year) (Selvestrel et al., 2022). The duration of the current subchronic study was 60 days to cover the duration of one complete spermatogenic cycle in rats (54 days) (Perrard et al., 2016). In addition, sperms can survive for 60 days in the cauda epididymis (Sevim et al., 2023). All doses were given to rats by oral gavage. The study groups were as follows:

Group (1) (Control): Distilled water (vehicle of acetamiprid) (0.5 ml/day, gastric gavage).

Group (2) (Oil): Corn oil (vehicle of vit E and Co Q10) (0.3 ml/day, gastric gavage).

Group (3) (Vit E): Vit E (100 IU/kg/day, gastric gavage dissolved in 0.3 ml corn oil) (Zayman et al., 2022).

Group (4) (CoQ10): Co Q10 (75 mg/kg/day, gastric gavage dissolved in 0.3 ml corn oil) (Najaran et al., 2019).

Group (5) (ACE): ACE (25 mg/kg/day approximately equivalent to 1/9 of LD50 for oral dose dissolved in 0.5 ml distilled water, gastric gavage) (Arıcan et al., 2020).

Group (6) (ACE + Vit E): ACE (25 mg/kg/day dissolved in 0.5 ml distilled water, gastric gavage) + Vit E (100 IU/kg/day, gastric gavage in 0.3 ml corn oil).

Group (7) (ACE+CoQ10): ACE (25 mg/kg/day dissolved in 0.5 ml distilled water, gastric gavage) + CoQ10 (75 mg/kg/day, gastric gavage dissolved in 0.3 ml corn oil).

Group (8) (ACE+ Vit E+CoQ10): ACE (25 mg/kg/day dissolved in 0.5 ml distilled water, gastric gavage) + Vit E

(100 IU/kg/day, gastric gavage dissolved in 0.3 ml corn oil) + CoQ10 (75 mg/kg/day, gastric gavage dissolved in 0.3 ml corn oil).

Rats were weighed at the beginning of the study and final body weights were recorded at the end of the study. After 60 days of exposure, fasted rats were sacrificed after euthanasia with intraperitoneal administration of 50 mg/kg ketamine hydrochloride (Ketalar®), and (10 mg/kg xylazine hydrochloride) (Zayman et al., 2022). Animals were sacrificed by cervical dislocation. Following the decapitation procedure, the testes and cauda epididymides of the rats were removed from the corpse and cleaned from the adipose and connective tissues (Trošić et al., 2013). Both testes were weighed and recorded as testicular weight.

Sperm collection and analysis of sperm parameters

For assessing the epididymal sperm counts; the caudal epididymis was minced in 5 mL of 0.9 % NaCl and each sample was properly mixed using a shaker for 10 min. The epididymal sperm suspension was incubated at 20 ◦C for 2 min. An alkaline solution containing eosin and 35 % formalin was used for supernatant dilution (1:100). A Neubauer hemocytometer was used to count sperm under a light microscope (200 ×), and the count is expressed in millions per ml of the epididymal suspension (Yokoi et al., 2003, Kong et al., 2017). Sperm motility is classified to: A, rapid progressive motility; B, slow progressive motility; C, non-progressive motility; or D, no motility) (Kong et al., 2017). In the present study, any type of sperm motility was considered. The percentage of total sperm motility was determined using a high-magnification (400 ×) light microscope (Kong et al., 2017).

Morphological assessment was carried out with 3 spreads for each sample and 200 sperm were counted for each preparation. The sperms were classified as normal and abnormal which included any of the following (headless, detached head, flattened head, pinhead, bent neck, bent tailed, coiled tail and multiple abnormalities) (Arıcan et al., 2020). Sperm viability was assessed by the eosin Y stain (Ibrahim et al., 2020)

Blood sampling and Serum Hormonal Assay

Blood samples were collected by cardiac puncture and kept at room temperature for 30 min. After coagulation, blood samples centrifugation at (4000 r.p.m / 4 0C) for 15 min to obtain serum. The serum was preserved at -80°C until used for hormonal assay. Serum luteinizing hormone (LH) and serum testosterone were assayed by using Cobas e411 analyzer in accordance with the manufacturer's protocols of the utilized kits (Elecsys LH Kit, Cobas, Roche diagnostic, USA) and (ab285350 – Mouse/Rat Testosterone ELISA Kit, abcam, China); respectively. The results were expressed as milli-international units per milliliter (mIU/mL) for serum LH, while as results were expressed as nanograms per milliliter (ng/ml) for serum testosterone

Testicular homogenate preparation and oxidative stress evaluation

The right testes were perfused in phosphate buffered saline (PBS) (pH 7.4) containing (0.16 mg/ml) heparin to remove blood clots. Each testicular tissue sample homogenized in 5-10 ml cold buffer (i,e, 50-10 mM potassium phosphate PH 7.5, 1mM EDTA) per gram tissue. Each sample of testicular homogenates was centrifuged at (4000 r.p.m/4 °C) for 15 min, and the

supernatant layer stored at -80^{o}C until used for oxidative stress biochemical evaluation. Colorimetric methods were used for assessing the oxidative stress biomarkers in testicular tissue homogenate and the used commercial kits were purchased from (Bio-diagnostic®, Giza, Egypt). The super oxide dismutase (SOD) activity, the reduced Glutathione (GSH) concentration and the malondi-aldehyde (MDA) concentration were determined according to the methods of (Beutler et al., 1963, Nishikimi et al., 1972; Ohkawa et al., 1979) respectively. The results expressed for SOD, GSH and MDA as unit per gram of testicular tissue homogenate (U/gm tissue), milligram per gram of testicular tissue homogenate (mg/gm tissue) and nanomole per gram of testicular tissue homogenate (nmol/g. tissue) respectively.

Histopathological Examination:

Left testes were kept for histopathological and immuno-histo-pathological analyses by immersion in 10% neutral buffered formalin for 48 h preparing for staining. Paraffin sections of five microns thickness from the testicular tissue were cut and stained by haematoxylin and eosin (H&E) for histological examination by using a light microscope (Olympus model BX53, Tokyo, Japan) equipped with a digital camera (Toucan type BX53, Japan).

Immunohistochemical investigation of caspase-3 and TNF-α in the testes:

Paraffin sections (4 µm thick) were deparaffinized in xylene and rehydrated with 0.03% H2O2 to block the endogenous peroxidases. After antigen retrieval in a microwave for 20 minutes with a pH-neutral sodium citrate buffer, the antigen was blocked with 5% bovine serum albumin in tris buffered saline. The sections were then treated with a primary antibody against caspase-3 (ab184787, 1: 1000 dilution) and TNF-α (ab307164, 1: 200 dilution) for an additional overnight period at 4 ° C. The ABC kit was used to detect the reaction according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). The sections were then dried, mounted with a synthetic glue medium, and counterstained with haematoxylin, then analysis of tissue sections was analysed by light microscopy (Hassan et al., 2022).

Images were taken using a digital camera (Toucan type BX53, Japan) linked to a computer and a light microscope (Olympus model BX53, Tokyo, Japan). For each rat in each group, the testes were examined in five randomly placed, 4 µm thick slices using a 40x lens and a 400x magnification (area: 0.071mm2). The area fraction of caspase-3 and TNF-α immune expression was the estimated parameter. Image-j (Fiji) was used for computerized image analysis. Brownish coloration was indicative of immune expression. Three separate-coloured images were created using a colour deconvolution plug-in with the H-DAB vector as the chosen colour: green, brown, and blue. The DAB images, which are coloured brown, were calibrated by determining the area fraction (Hassan et al., 2022).

Statistical Analysis

Data were analysed by using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 26) and data normality was approved by using One-sample Kolmogorov-Smirnov test. Continuous variables were presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) test followed

by Tukey-Kramer multiple comparisons post hoc test were used for comparing the mean values between the studied groups. The threshold of significance was fixed at 5% level (p-value). The results were considered significant when the probability of error is less than 5% ($p < 0.05$).

Results

Animals in all groups were included in the study; as no reported mortality or morbidity among all groups during the whole period of the study.

As shown in (table (1); the (ACE) group had significantly decreased final body weight and testicular weight compared to the control group. (ACE +Vit E), (ACE+CoQ10) and (ACE +Vit E+CoQ10) groups had significantly increased body weight compared to (ACE). Only rats of (ACE +Vit E+CoQ10) groups achieved the control group values. Testicular weight of (ACE +Vit E) and (ACE+CoQ10) groups were insignificantly higher compared to (ACE) group. The (ACE +Vit E+CoQ10) group had significantly higher testicular weights compared to (ACE) and achieved control group values. Final body weight and testicular weight of rats in Oil, (Vit E), (CoQ10) groups showed no significant difference compared to control group.

Table (1): Comparing the effect of ACE and co-administration of Vit E and/or CoQ10 with ACE on final body weight and testicular weight of adult spurge dewily rats (n=48).

Variables	Control (n=6)	Oil (n=6)	Vit E (n=6)	CoQ10 (n=6)	ACE (n=6)	ACE + Vit E (n=6)	ACE + CoQ10 (n=6)	ACE+VitE+ Q10 (n=6)	One-Way ANOVA test
Final Body weight (g)	345 ± 5 a	346.67 ± 7.64 a	345 ±15 a	345 ± 10 a	270 ± 10 b	330 ± 5 c	310 ± 10 c	360 ±1 0 a	F= 27.28 p < 0.001*
Testicular weight (g)	3.53±0.32 a	3.70 ± 0.26 a	3.80 ± 0.20 a	3.80 ± 0.20 a	2.86 ± 0.15 b	3.23 ± 0.20 b	3.20 ± 0.10 b	3.90 ± 0.10 a	F= 8.74 p < 0.001*

F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.
Data expressed as mean ± standard deviation (SD).
Values shown with different letters (a, b and c) are statistically different at $P < 0.05$*
Vit E: Vitamin E, CoQ10: Co enzyme Q10, ACE: Acetamiprid, n: number of rats in group, p: significance, g: gram.

Hormonal assay results revealed that, (ACE) group had significantly lower LH and testosterone levels compared to the control group. The (ACE+ Vit E), (ACE+CoQ10) and (ACE+VitE+Q10) groups had significantly higher LH levels compared to (ACE) group. In addition, (ACE+VitE+Q10) group had significantly higher LH levels compared to both (ACE+ Vit E) and (ACE+CoQ10) groups. Testosterone levels of (ACE+ VitE+Q10) group were statistically significantly higher than those of (ACE) group. Both (ACE+ Vit E) and (ACE+ CoQ10) groups had insignificantly higher testosterone levels compared to (ACE) group. Both LH and testosterone levels were significantly lower than control levels in (ACE+ Vit E), (ACE+CoQ10) and (ACE+ VitE+Q10) groups. Oil, (Vit E) and (CoQ10) groups had no significant differences with control group as regard LH and testosterone levels (Table 2).

Table (2): Comparing the effect of ACE administration and co-administration of Vit E and/or CoQ10 with ACE on serum Testosterone and luteinizing hormone in adult spurge dewily rats (n=48).

Variables	Control (n=6)	Oil (n=6)	Vit E (n=6)	CoQ10 (n=6)	ACE (n=6)	ACE + Vit E (n=6)	ACE + CoQ10 (n=6)	ACE+VitE+ Q10 (n=6)	One-Way ANOVA test
Luteinizing hormone(LH) (mIU/mL)	2.35 ± 0.11[a]	2.30 ± 0.01[a]	2.53 ± 0.02[a]	2.49 ± 0.13[a]	1.13 ±0.03 [b]	1.39 ± 0.05 [c]	1.32 ± 0.04[c]	1.62 ± 0.12 [d]	F= 159.282 p < 0.001*
Testosteron (ng/ml)	4.80 ± 0.20 [a]	4.90 ± 0.40 [a]	5.43 ± 0.20 [a]	5.30 ± 0.19 [a]	1.80 ± 0.02 [b]	2.41 ± 0.05[b]	2.20 ± 0.03[b]	3.0± 0.36[c]	F= 134.870 p < 0.001*

F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.
Data expressed as mean ± standard deviation (SD).
Values shown with different letters (a, b, c and d) are statistically different at $P < 0.05$*
Vit E: Vitamin E, CoQ10: Co enzyme Q10, ACE: Acetamiprid, n: number of rats in group, p: significance, mIU/mL milli-int units per milliliter, ng/ml: nanograms per milliliter.

As regard semen analysis results (Table 3) illustrated that, (ACE) group significantly decreased sperms count and percentage of sperms motility in line with significant increase in percentage of dead sperms and percentage of abnormal sperms compared to control group. On the other hand, (ACE +Vit E) group, (ACE+ CoQ10) group and (ACE+VitE+CoQ10) group showed significant increase in sperm count and percentage of sperms motility as well as significant decrease in percentage of dead sperms and percentage of abnormal sperms compared to (ACE) group. In addition, both (ACE+ vit E) group and (ACE+VitE+CoQ10) group had significantly higher sperm motility compared to (ACE+CoQ10) group. The (ACE+VitE+CoQ10) group had significantly lower percentage of dead sperms compared to both (ACE+ Vit E) group and (ACE+CoQ10) group. Oil, (VitE), (CoQ10) groups showed no significant difference compared to control group as regard sperm count as well as percentage of both dead sperms and abnormal sperms. Both (Vit E) and (CoQ10) groups showed significantly higher sperm motility compared to control and oil groups.

Table (3): Comparing the effect of ACE and co-administration of Vit E and/or CoQ10 with ACE on semen parameters in adult spurge dewily rats (n=48).

Variables	Control (n=6)	Oil (n=6)	Vit E (n=6)	CoQ10 (n=6)	ACE (n=6)	ACE + Vit E (n=6)	ACE + CoQ10 (n=6)	ACE + VitE + Q10 (n=6)	One-Way ANOVA test
Sperm Count (million / ml)	110 ± 10.0 [a]	116.33± 7.09 [a]	125.00± 1.0 [a]	127.33 ± 2.517 [a]	30.00 ± 10.0 [b]	64.67 ± 5.03 [c]	68.00 ± 6.0 [c]	80.00 ± 5.0 [c]	F= 85.094 p < 0.001*
Sperm motility %	42.67 ± 2.52 [a]	42.00 ±1.0 [a]	52.33 ± 3.05[b]	47.67 ± 2.51 [b]	12.33 ± 2.50 [c]	27.67 ± 2.0 [f]	22.33 ± 2.17 [d]	32.33 ± 2.52 [f]	F= 92.481 p < 0.001*
Sperm Abnormalities %	11.67 ± 1.5 [a]	11.33 ± 1.53 [a]	11.67 ± 1.52 [a]	12.33 ± 1.58 [a]	30.67 ± 2.08 [b]	24.33 ± 1.5 [c]	24.00 ± 2.0 [c]	20.33 ± 1.5 [c]	F= 61.115 p < 0.001*
Dead sperms%	22.33 ±1.5 [a]	21.33 ±1.53 [a]	20.00 ±1.00 [a]	20.00 ± 2.00 [a]	47.67 ± 3.51 [b]	40.00 ±1.0 [c]	39.00 ± 1.0 [c]	32.67 ± 2.08 [d]	F= 100.798 p < 0.001*

F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.
Data expressed as mean ± standard deviation (SD).
Values shown with different letters (a, b, c, d and f) are statistically different at $P < 0.05$*
Vit E: Vitamin E, CoQ10: Co enzyme Q10, ACE: Acetamiprid, %: percentage, n: number of rats in group, p: significance, ml: m

Oxidative stress evaluation in testicular tissues of (ACE) group showed significant decrease of the antioxidants SOD and GSH; parallel to significant increase of oxidative stress marker MDA compared to control group. On comparison with (ACE) group; (ACE + vit E), (ACE + CoQ10) and (ACE+VitE+CoQ10) groups showed significant improvement in the form of significantly increased SOD and GSH as well as significantly decreased MDA. Only GSH achieved the control levels in (ACE+ vit E), (ACE+CoQ10) and (ACE+VitE+CoQ10) groups. As regard testicular SOD, GSH and MDA; there was no statistically significant difference between (ACE+ vit E) group, (ACE+CoQ10) group and (ACE+ VitE+ CoQ10) groups. In addition, Oil, (VitE) and (CoQ10) groups had no significant difference in SOD, GSH and MDA testicular levels compared to the control group (Table 4).

Table (4): Comparing the ACE induced testicular oxidative stress and the antioxidant effects of co-administration of Vit E and / or CoQ10 with ACE in adult spurge dewily rats (n=48).

Variables	Control (n=6)	Oil (n=6)	Vit E (n=6)	CoQ10 (n=6)	ACE (n=6)	ACE + Vit E (n=6)	ACE + CoQ10 (n=6)	ACE+VitE +Q10 (n=6)	One-Way ANOVA test
Testicular SOD (U/g. tissue)	188.53 ±1.23 [a]	188.70 ± 8.80 [a]	199.13 ± 1.00 [a]	197.77 ± 0.80 [a]	88.20± 2.43 [b]	144.50 ± 2.23 [c]	137.64 ± 6.46 [c]	150.47 ±5.70 [c]	F= 218.021 p < 0.001*
Testicular GSH (mg/g. tissue)	3.35 ± 0.39 [a]	2.77 ± 0.25 [a]	3.25 ± 0.03[a]	2.48 ± 1.15[a]	0.80 ± 0.19 [b]	2.30 ± 0.30 [a]	2.47± 0.35[a]	2.60 ± 0.40[a]	F= 7.542 p < 0.001*
Testicular MDA (nmol/g. tissue)	10.50 ± 0.50 [a]	11.03±0.25 [a]	11.53 ± 0.35 [a]	10.00± 0.95 [a]	45.47± 4.86 [b]	26.67 ± 2.14 [c]	29.50 ± 0.36 [c]	25.27 ± 2.40 [c]	F= 110.354 p < 0.001*

*F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test. Data expressed as mean ± standard deviation (SD). Values shown with different letters (a, b, and c) are statistically different at P <0.05**

Vit E: Vitamin E, CoQ10: Co enzyme Q10, ACE: Acetamiprid, (SOD) Superoxide dismutase, (GSH) Reduced glutathione, (MDA) malondialdehyde, (U/g tissue) unit per gram of tissue homogenate, (mg/g. tissue) milligram per gram of tissue homogenate, (nmol/g. tissue) nanomole per gram of tissue homogenate, n: number of rats in group, p: significance.

The histological evaluation of testes in the control group (Figures 1- A & 1-B) revealed normal architecture in the form of packed seminiferous tubules lined by germinal epithelium and with narrow clear lumens. The germinal epithelium contained pyramidal-shaped Sertoli cells and multiple layers of spermatogonia which were supported by a thin basement membrane. The interstitial spaces between the seminiferous tubules were narrow and contained Leydig cells. The oil group, (Vit E) group and (CoQ10) group had normal testicular architecture like the control group.

The (ACE) group (Figures 1-C & 1-D) showed marked degenerative changes in the form of distorted and disorganized seminiferous tubules with few layers of separated spermatogonia and widened lumen that contained desquamated cells. Also, the interstitial spaces showed marked widening as compared to the control group.

The (ACE + Vit E) group (Figures 1-E & 1-F) and (ACE+CoQ10) group (Figures 1-G & 1-H); showed variable histo-pathological alterations in the form of irregular seminiferous tubules with thinning of their germinal epithelium and widened interstitial spaces. Most of the tubules contained luminal desquamated cells. The (ACE+ Vit E+ CoQ10) group (Figures 1-I & 1-J) showed a picture of regenerated seminiferous tubules which appeared more packed with narrow interstitial spaces and multiple layers of germinal epithelium. Less marked luminal desquamated cells were seen in the lumens.

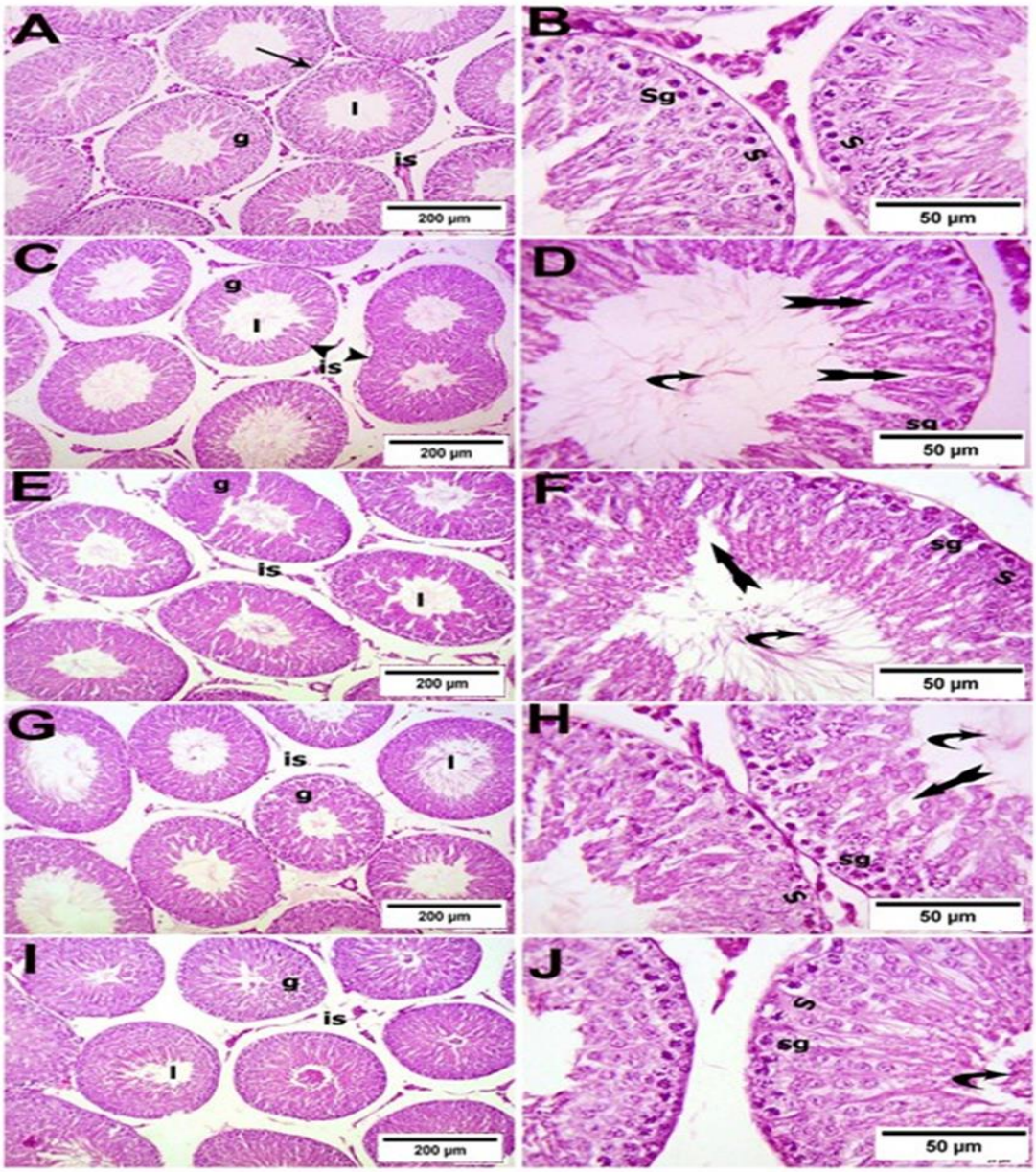

Fig. (1): Representative photomicrographs of hematoxylin and eosin-stained testicular sections from different experimental groups. (A & B): represents the control group and showed normal testicular architecture, displayed packed seminiferous tubules with narrow clear lumens (l) containing Leydig cells and lined by germinal epithelium (g) supported by a thin basement membrane (arrow). The germinal epithelium contained pyramidal-shaped Sertoli cell (S) and multiple layers spermatogonia (sg). The interstitial spaces between the seminiferous tubules were narrow (is). (C & D) represents the (ACE) group and showed distorted and disorganized tubules separated by widened interstitial spaces (between arrowheads). The germinal epithelium was thin with a few layers of spermatogonia (sg) which appeared separated by leaving spaces (tailed arrows). The tubular lumens (l) are widened and contain desquamated cells in lumen (curved arrow). (E & F) represents (ACE+ Vit E) group and (G & H) represents (ACE+CoQ10) group both groups showed variable histopathological alterations the form of irregular seminiferous tubules with thinning of their germinal epithelium and widened interstitial spaces (is). Most of the tubules contain luminal desquamated cells (curved arrow). (I & J): represents (ACE+VitE+CoQ10) group which showed a picture of regenerated tubules which appeared more packed with narrow interstitial spaces (is) and multiple layers of geminal epithelium (g). Less marked luminal desquamated cells (curved arrow) were seen in the lumens (H & E; A, C, E, G, I x 100 and B, D, F, H, J x 400).

The immunohistochemical evaluation of the control group revealed negative immune reaction to both Caspase-3 (Figure 2-A) and TNF α (Figure 3-A) in testicular tissues. Also, testicular tissues of oil group, (Vit E) group and (CoQ10) group showed negative reaction to Caspase-3 and TNF-α with no statistically significant difference compared to the control group (Table 5).

As shown in (Figures 2- B, 2-C, 2-D & 2-E) and (Table 5); Caspase-3 reaction was the strongest in (ACE) group compared to other groups. The (ACE+ Vit E) group and (ACE+CoQ10) group showed significant decrease in Caspase-3 expression compared to (ACE) group although this improvement did not reach the control levels; while as the (ACE+ Vit E+ CoQ10) group showed marked decrease in caspase-3 reaction that achieved the control levels.

As illustrated in (Figures 3-B, 3-C, 3-D & 3-E) and (Table 5); (ACE) group had the strongest reaction to TNF- α compared to other groups. In addition, (ACE+ Vit E) group, (ACE+CoQ10) group and (ACE+ Vit E+ CoQ10) group showed significantly decreased TNF-α expression compared to (ACE) group, although this anti-inflammatory effect didn't reach the control levels.

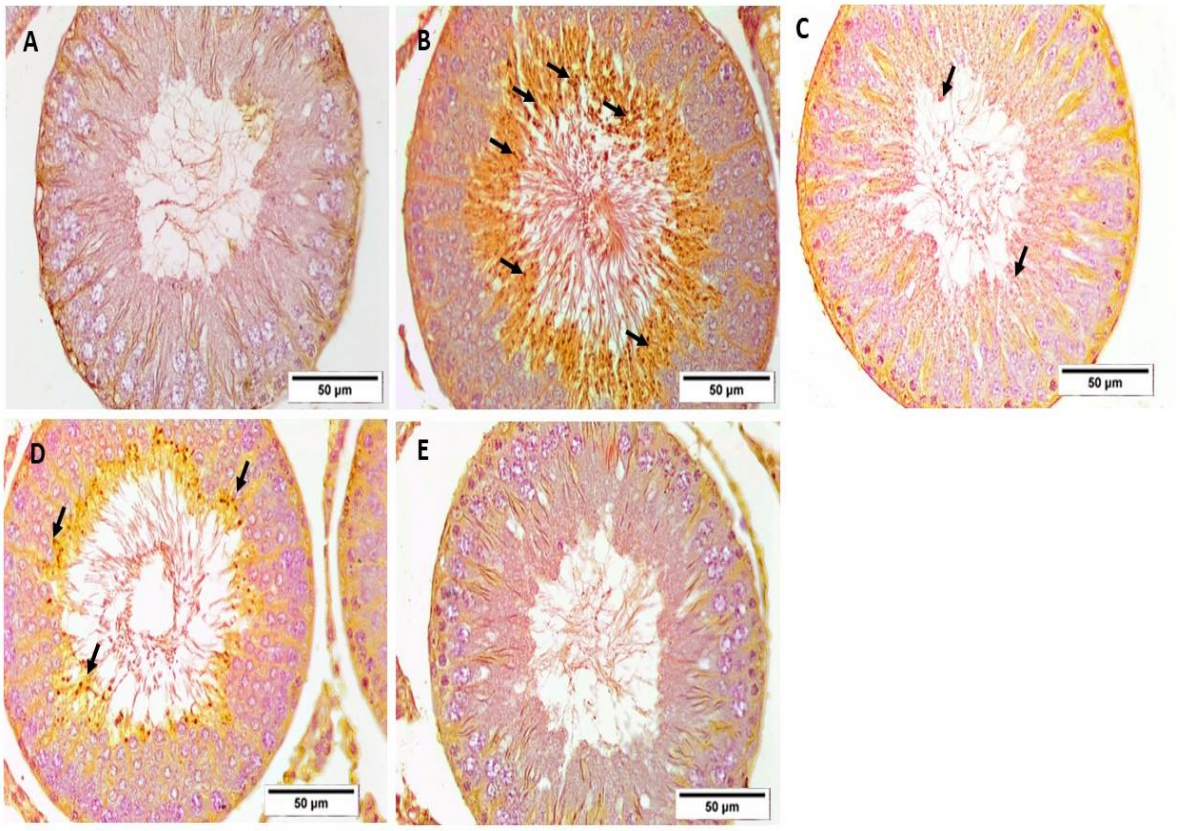

Fig. (2): Representative photomicrograph of immune-histochemical caspase-3 expression in testicular tissues from different experimental groups. (A): showed negative Caspase-3 reaction in control group. (B): showed the strongest positive reaction in (ACE) group. (C) and (D): showed mild positive Caspase-3 reaction in (ACE+ Vit E) and (ACE+CoQ10) groups, respectively. (E): showed faint Caspase-3 reaction in (ACE+VitE+CoQ10) group. The arrows pointed to the positive caspase-3 reaction. Image magnification= x 400.

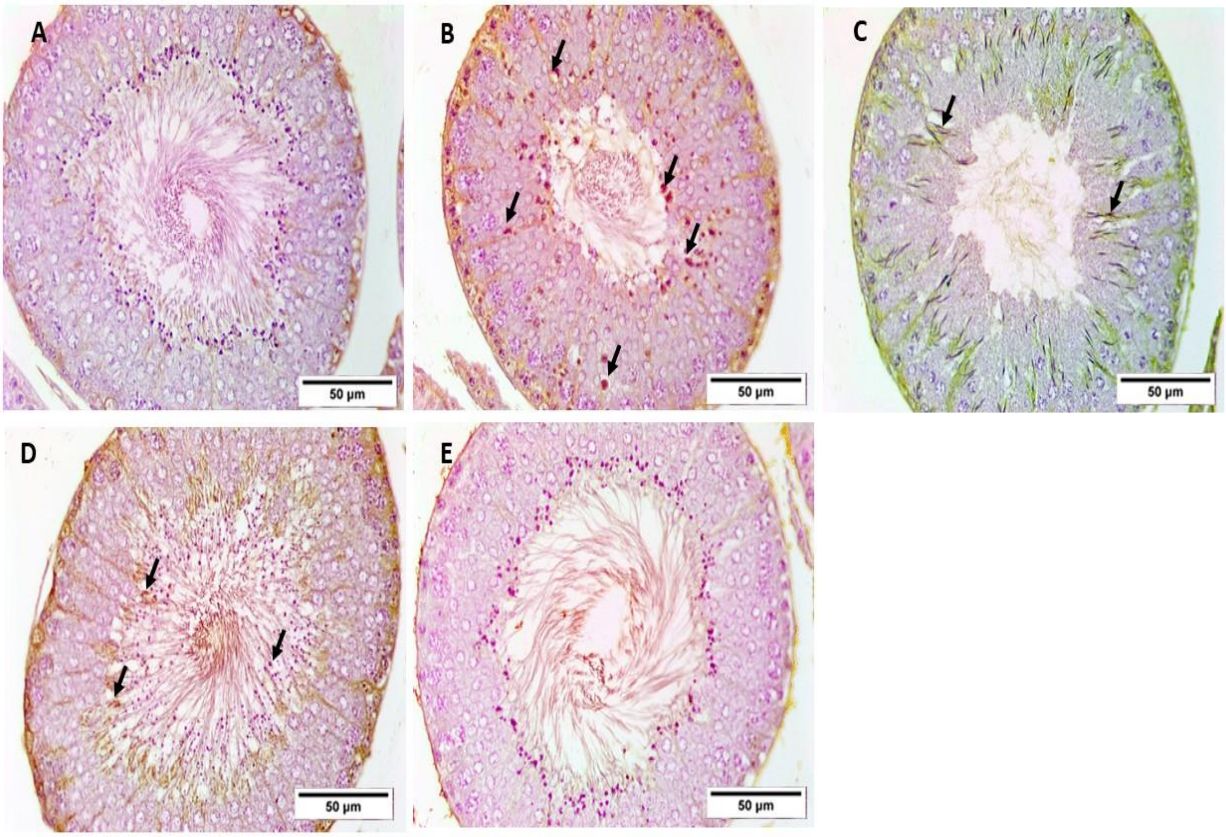

Fig. (3): Representative photomicrograph of immune-histochemical TNF-α expression in testicular tissues from different experimental groups. (A): showed negative TNF-α reaction in control group. (B): showed the strongest TNF- α positive reaction in (ACE) group. (C) and (D): showed mild positive TNF- α reaction in (ACE+ Vit E) and (ACE+CoQ10) groups, respectively. (E): showed faint TNF α reaction in (ACE+VitE+CoQ10) group. The arrows pointed to the positive TNF-α reaction. Image magnification= x 400.

Table (5): Comparing the testicular expression of Caspase-3 as well as TNFα (area %) between the studied groups (n=48).

Variables	Control (n=6)	Oil (n=6)	Vit E (n=6)	CoQ10 (n=6)	ACE (n=6)	ACE + Vit E (n=6)	ACE + CoQ10 (n=6)	ACE+VitE +Q10 (n=6)	One-Way ANOVA test
Caspase-3	20.77 ± 1.01[a]	21.56 ±1.0[a]	20.50 ±1.0[a]	19.50 ± 1.01[a]	71.45 ± 11.4[b]	46.65 ± 5.5[c]	49.2 ± 5.8[c]	32.87 ± 6.5 [a]	F= 39.291 p < 0.001*
TNF-α	13.35 ± 2.60[a]	12.80 ±1.41[a]	12.33 ± 0.70[a]	11.03 ± 0.50[a]	67.27 ± 8.2[b]	37.89 ± 10.5[c]	38.41 ± 8.1[c]	25.98 ± 9.7[c]	F= 43.474 p < 0.001*

F: One-way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.
Data expressed as mean ± standard deviation (SD).
Values shown with different letters (a, b, and c) are statistically different at P < 0.05*
Vit E: Vitamin E, CoQ10: Co enzyme Q10, ACE: Acetamiprid, n: number of rats in group, p: significance, TNF α necrotic factor alpha.

Discussion

The ACE is one of the most widely used nicotinamide pesticides (Zuščíková et al., 2023). Various human and experimental studies concerned with ACE induced male reproductive toxicity (Neghab et al., 2014; Arıcan et al., 2020). Both Vit E and CoQ10 are natural agents which have beneficial effects on male reproduction (Oyovwi et al., 2022 and Zayman et al., 2022). The current wok aimed at investigating protective effect of Vit E and/or CoQ10 against ACE male reproductive toxicity.

In accordance with Zayman et al. (2022); the current ACE exposed rats had significantly lower serum levels of both testosterone and LH in compared to control group, despite the number of Leydig cells was not decreased in ACE group compared to controls. Accordingly, EL‑Hak et al. (2022) reported that ACE decreased LH serum levels in a dose dependent manner in adult male rats. The testosterone deficiency in ACE intoxicated animal models could be assumed to ACE central effect on hypothalamus-pituitary-testicular axis by decreasing the pituitary secreted LH. Besides, Arıcan et al., (2020) found that testosterone deficiency was linked to decreased serum cholesterol level, a precursor for testosterone biosynthesis, in ACE exposed rats.

Terayama et al. (2018) reported the link between the nicotine like action of ACE and the inhibition of testosterone synthesis; as the ACE exposed mice showed decreased Leydig cells expression of nicotinic acetylcholine receptors subunit genes (nAChRs α4, α7), in line with decreased testosterone- metabolism genes [the steroidogenic acute regulatory protein (StAR), cytochrome P450, family 11, subfamily a, polypeptide 1 (CYP11A1), and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase cluster (HSD3B)]. The StAR protein is essential for mitochondrial transportation of cholesterol, where cholesterol is converted to pregnenolone by CYP11A1. In the endoplasmic reticulum, HSD3B converts the pregnenolone to progesterone which is catalyzed finally to testosterone (Kong et al, 2017).

Inconsistent with our results; (Zhang et al.2011, Kong et al.2017 and Arıcan et al., 2020) demonstrated increased LH serum levels, in line with decreased testosterone serum levels, in ACE intoxicated rats. Kong et al. (2017) explained the increased LH levels by the compensatory feedback response in the hypothalamus-pituitary-testicular axis to the decreased testosterone. This discrepancy could be assumed to variability in ACE induced hormonal changes according to difference in doses and duration of exposure to ACE (EL‑Hak et al., 2022).

Similar to Zhang et al. (2011), significant reduction in body as well as testicular weights were observed in ACE treated rats in the current work. Mondal et al. (2015) attributed the decreased body weight to reduced feed intake in ACE treated animals compared to control; due to anorectic effect of ACE. In consistent with our findings; (Devan, et al 2015 and Mosbah et al., 2018) attributed the decreased testicular weight and increased body wight in ACE exposed model to reduced testosterone as well as pituitary FSH and LH. In partial agreement; (Terayama et al., 2018; Arıcan et al., 2020) reported deceased body weight while as the testicular weight was not affected in ACE exposed rats. Different from our results, Toghan et al. (2022) found that ACE treated group had significantly increased the testicular weight in line with decreased body weight. The increased testicular weight was attributed to oedema and congestion; while as, in late stages, the decreased testicular weight occurred due to marked degenerative changes (Rachid et al., 2013).

The current results agreed with (Kong et al, 2017, Kenfack et al., 2018; Mosbah et al., 2018; Guiekep et al., 2019; Toghan et al., 2022) who approved the association between induction of testicular oxidative stress and the decreased semen quality in ACE exposed animal models. Interestingly, meticulous balance of free oxygen radicals is needed for spermatogenesis. The integrity of cell membrane is essential for motility and vitality of mature sperms (Toghan et al., 2022).

Oxidative stress refers to the imbalance between the increased free oxygen radicals and the decreased enzymatic (e.g., SOD) and /or non-enzymatic (e.g., GSH) free radicals' scavengers (Mosbah et al., 2018). Oxidative stress in Leydig cells decreases the mitochondrial dependent steps of testosterone biosynthesis (Yilmaz et al., 2018). Free oxygen radicals lead to DNA damage and lipid peroxidation of cell membrane which affects both spermatogenesis and sperm parameters (Kenfack et al., 2018). In partial agreement with us; Humann-Guilleminot et al. (2019) reported that in birds exposed to ACE, the GSH and MDA levels were not affected, while as sperm count and SOD level were decreased. This discrepancy could be assumed to species, dose and duration differences.

In harmony with (Zhang et al., 2011; Rachid et al., 2013; Kong et al., 2017; Terayama et al., 2018; Zayman et al. 2022), the current histological examination of ACE group revealed testicular degenerative changes and impaired spermatogenesis. Kong et al. (2017) explained the impaired spermatogenesis by ACE induced testosterone deficiency as well as ACE induced oxidative stress. Like our findings; (Zayman et al., 2022; Sevim et al., 2023) observed increased testicular Caspase-3 expression in ACE exposed rats. Apoptosis represents the programmed cell death. Although physiological apoptosis is essential for eliminating abnormal spermatogenic cells, excessive apoptosis leads to impaired spermatogenesis (Zayman et al. 2022). The process of apoptosis is executed either through the mitochondrial pathway and/or the extrinsic pathway which is initiated via death receptors on the cell membrane. Caspases are cysteine ‑ aspartic proteases which act as the initiators of both apoptotic pathways (Sevim et al., 2023). Once Caspase-3 is activated, the apoptosis process will be irreversible (D'Arcy, 2019). Accordingly, Zhang et al. (2011) reported that oxidative stress activated the proapoptotic pathway p38 mitogen-activated protein kinase (MAPK) by phosphorylation in testes of ACE exposed mice. In addition, free radicals change the mitochondrial membrane permeability with subsequent activation of apoptotic mitochondrial pathway (Kong et al., 2017).

Furthermore, decreased cell proliferation biomarkers were considered as possible mechanism of ACE impaired spermatogenesis, as Terayama et al. (2018) reported decreased marker of proliferation Kiel 67 (Ki67) and topoisomerase II alpha (Top2a) in testes of ACE intoxicated mice. Arıcan et al. (2020) reported decreased proliferative cell nuclear antigen expression in line with increased apoptotic index in seminiferous tubules of ACE exposed rats.

Furthermore, the current ACE administration significantly increased TNFα expression as a marker of testicular inflammation. In line with our results, the ACE exposed animal models showed increased expression of inflammatory mediators [TNFα, interleukin-1β (IL-1β), and nuclear factor kappa B-(NF-κB)] in cerebral cortex (Albrakati , 2024), in liver tissue (Phogat et al., 2023) and in renal tissue (Alhusaini et al., 2019). Shakthi Devan et al. (2015) approved the immune toxicity due to ACE exposure in the form of macrophages functions disruption with subsequent release

of proinflammatory cytokines. In general, oxidative stress and inflammation form a close circuit. Free radicals associated with increased (NF-κB) which recruit inflammatory cells. In turn, inflammatory mediators as TNF-α can induce more production of free radicals (Alhusaini et al., 2019)

In agreement with (Zhang et al., 2011; Kong et al., 2017; Zayman et al., 2022), the present co-administration of Vit E with ACE significantly improved all sperm parameters and testicular degenerative changes in line with Vit E antioxidant effects. Like our results, Zayman et al., (2022) reported significant decreased testicular expression of proapoptotic Caspase-3 on using Vit E with ACE. In line with our results, adjuvant treatment with Vit E decreased testicular inflammatory mediators including TNFα, in lead intoxicated rats (Khafaji, 2023) and in monosodium glutamate exposed rats (El Kotb et al., 2020). In addition, the present administration of Vit E with ACE led to significantly increased serum LH and insignificantly increased serum testosterone compared to ACE treated group. Accordingly, Kong et al. (2017) used Vit E as adjuvant with ACE and reported increased serum testosterone via increased expression of testosterone - metabolism genes (CYP11A1, STAR, and HSD3B) at both the protein and mRNA levels. Karanth et al. (2003) demonstrated increased pituitary LH, in response to Vit E supplement in healthy rats, via increased the hypothalamic luteinizing hormone-releasing hormone (LHRH).

Current findings revealed that co-administration of CoQ10 with ACE improved all the investigated semen parameters with remarkable CoQ10 antioxidant effects. On reviewing literature, no previous studies investigated CoQ10 ameliorating effects against ACE induced male reproductive toxicity. Our results agreed with, several studies that used COQ10 as adjuvant therapy against testicular injury in animal models of the following conditions: high magnetic fields (Ramadan et al., 2002), oxytetracycline toxicity (Oda et al., 2018), varicoceles (Najaran et al., 2019), sodium arsenate toxicity (Sharma et al., 2021) and high fat diet (Allam et al, 2022). In the present findings, (ACE+CoQ10) group had significantly increased LH and insignificantly increased testosterone levels compared to ACE group. Unlike our results, El‑Sherbiny et al. (2022) reported significantly increased testosterone with COQ10 adjuvant treatment in heat induced testicular injury.

Present data demonstrated anti-inflammatory and antiapoptotic effects of CoQ10 with mitigation of ACE induced degenerative changes in testicular tissue. Accordingly, CoQ10 cotreatment decreased expression of TNFα, nuclear factor-κB and Caspase-3 in testicular tissues of arsenic intoxicated rats (Fouad et al., 2011). Also, antioxidant effects of COQ10 suppressed the expression of inflammatory markers (TNFα and interleukin-1 beta), attenuated expression of proapoptotic factors (Bax and Caspase-3) and induced expression of anti-apoptotic (Bcl2) in testicular tissues of lead intoxicated rats (El-khadragy et al., 2020).

Interestingly, the exact protective dose of COQ10 in male reproduction is still not established either in human or animal studies (Salvio et al., 2021). Various doses of CoQ10 had been used to achieve its male reproductive protecting effects e.g. (75mg/kg/day) which was used in current work and was used by (Najaran et al., 2019, Allam et al, 2022) for (60 days and 3 months, respectively. Higher dose of CoQ10 (200mg/kg/day) was used by (Ramadan et al., 2002) three times per week for 2 weeks, while as, lower dose (10 mg/kg/day) was used by (Oda et al., 2018) for 30 days.

In the current study combined usage of Vit E and CoQ10 had significant ameliorating effects against ACE induced effects on (body weight, testicular weight, serum testosterone, serum LH, sperm motility, number of dead sperms and caspase-3 expression) compared to individual usage of each agent. This finding can be explained by the synergistic effect of concurrent Vit E and CoQ10 usage; as well as the ability of CoQ10 to act as Vit E precursor (Ognjanović et al., 2010). Notably, no significant differences were observed between the protective effects of vit E, CoQ10 and their combined usage as regard oxidative stress markers, sperm count, sperm abnormal forms and TNFα expression. In consistent with our findings, the combined use of Vit E and CoQ10 had more effective testicular antioxidant effects compared to individual administration of each agent in arsenic intoxicated rats (Sharma et al., 2021), and in cadmium intoxicated rats (Ognjanović et al., 2010). Different from our data; (Arda et al., 2021) found that CoQ10 had more remarkable testicular antioxidant effect than Vit E against ischemia/reperfusion injury in rats. This discrepancy could be assumed to variability of agents with different mechanisms of actions that were responsible for testicular injury in each study. Moreover, in current study, Vit E was superior to CoQ10 in improving sperm motility in ACE exposed rats. Similarly, Vit E showed more remarkable improving effects compared to melatonin against ACE male reproductive toxicity (Zayman et al., 2022). Similar to (Raouf and Taha, 2021); the current administration of Vit E and CoQ10 to healthy rats in (Vit E and CoQ10) groups, had significantly better sperm motility compared to control group, with no significant difference between them.

To conclude; the current study agreed with the previous studies concerned the ACE induced male reproductive toxicity. The present exposure to ACE was associated with impaired spermatogenesis and poor semen quality via several mechanisms including endocrine disruption as well as induction of testicular oxidative stress, inflammation and apoptosis. The usage of Vit E and /or COQ10 as adjuvant therapy with ACE was associated with ameliorating most of the ACE toxic effects. It was noted that the combined use of Vit E and CoQ10 was superior to the single use of each agent in increasing (body weight, testicular weight, sperm motility, sperm vitality, serum testosterone and serum LH) as well as decreasing testicular expression of Caspase-3.

Yet some of the investigated parameters failed to achieve control levels despite significant improvement compared to ACE group. The GSH achieved control levels with either single or combined use of Vit E and CoQ10; while as body weight, testicular weight and testicular Caspase-3 expression returned to control values with combined use of Vit E and CoQ10. Hence, the combined use of Vit E and CoQ10 could be considered in ameliorating ACE induced male reproductive toxicity. A possible limitation in the current work is that the ACE withdrawal effect, on the possibility of either amelioration or recovery of male toxicity, was not evaluated. Further investigations of other reported protective doses CoQ10 against ACE induced male reproductive toxicity alone or with Vit E and other antioxidants is highly recommended, in line with evaluation of other subcellular pathways e.g., endoplasmic reticulum stress and autophagy.

Conflicts of interest: The current work has no conflict of interest and did not receive any organization or financial support.

References

Albrakati, A. (2024): "The potential neuroprotective of luteolin against acetamiprid-induced neurotoxicity in the rat cerebral cortex". *Frontiers in Veterinary Science*, 11, pp.1-11**.** https://doi.org/10.3389/fvets.2024.1361792

Alhusaini, A.; Fadda, L.M.; Ali, H.M.; et al. (2019): "Mitigation of acetamiprid—induced renotoxicity by natural antioxidants via the regulation of ICAM, NF-kB and TLR 4 pathways". *Pharmacological Reports*, 71, pp. 1088-1094. https://doi.org/10.1016/j.pharep.2019.06.008

Allam, E.A.; Ibrahim, H.F.; Abdulmalek, S.A.; et al. (2022): "Coenzyme Q10 alleviates testicular endocrine and spermatogenic dysfunction induced by high‐fat diet in male Wistar rats: Role of adipokines, oxidative stress and MAPK/ERK/JNK pathway". *Andrologia*, 54(10), pp. e14544. https://doi.org/10.1111/and.14544

Arda, E.; Yuksel, I.; Akdere, H.; et al. (2021): "Contrary effects of coenzyme Q10 and vitamin E after testicular ischemia/reperfusion in a rat model validated with glucose metabolism imaging". *Urologia Journal*, *88*(1), pp. 56-63. https://doi.org/10.1177/0391560319882232

Arıcan, E.Y.; Gökçeoğlu Kayalı, D.; Ulus Karaca, B.; et al. (2020): "Reproductive effects of subchronic exposure to acetamiprid in male rats". *Scientific Reports*, 10(1), pp. 8985. https://doi.org/10.1038/s41598-020-65887-0

Beutler, E.; Duron, O. and Kelly, B.M. (1963): "Improved method for the determination of blood glutathione". *Journal of Laboratory and Clinical Medicine*; 61: pp. 882- 888 https://pubmed.ncbi.nlm.nih.gov/13967893/ .

D'arcy, M.S. (2019): "Cell death: A review of the major forms of apoptosis, necrosis and autophagy". *Cell Biology International*, 43(6), pp. 582-592. https://doi.org/10.1002/cbin.11137.

Devan, R.S.; Mishra, A.; Prabu, P.C.; et al. (2015): "Sub-chronic oral toxicity of acetamiprid in Wistar rats". *Toxicological & Environmental Chemistry*, 97(9), pp. 1236-1252. https://doi.org/10.1080/02772248.2015.1092542

Dukhnytskyi, V.; Sokolyuk, V.; Boiko, P.; et al. (2020): "The acute toxicity assessment of Mospilan RP and Actara 25 WG for White Mice*". Journal of World's Poultry Research*, 10(2), pp. 359-366. https://dx.doi.org/10.36380/jwpr.2020.41

El Kotb, S.M.; El-ghazouly, D.E.S. and Ameen, O. (2020): "The potential cytoprotective effect of Vitamin C and Vitamin E on monosodium glutamate-induced testicular toxicity in rats". *Alexandria Journal of Medicine*, *56*(1), pp. 134-147. https://doi.org/10.1080/20905068.2020.1804311

El‐Bialy, B.E.; Abd Eldaim, M.A.; Hassan, A.; et al. (2020): "Ginseng aqueous extract ameliorates

lambda ‐ cyhalothrin ‐ acetamiprid insecticide mixture for hepatorenal toxicity in rats: role of oxidative stress ‐ mediated proinflammatory and proapoptotic protein expressions". *Environmental Toxicology*, 35(2), pp. 124-135. https://doi.org/10.1002/tox.22848

EL-Hak, H.N.G.; Al-Eisa, R.A.; Ryad, L.; et al. (2022): "Mechanisms and histopathological impacts of acetamiprid and azoxystrobin in male rats". *Environmental Science and Pollution Research*, 29, pp. 43114–43125. https://doi.org/10.1007/s11356-021-18331-3

El-Khadragy, M., Al-Megrin, W. A., AlSadhan, N.A., et al. (2020): "Impact of coenzyme Q10 administration on lead acetate ‐ induced testicular damage in rats". *Oxidative Medicine and Cellular Longevity*, *2020*(1), p.p. 4981386. https://doi.org/10.1155/2020/4981386

El-Sherbiny, H.R.; Abdelnaby, E.A.; El-Shahat, K.H.; et al. (2022): "Coenzyme Q10 Supplementation enhances testicular volume and hemodynamics, reproductive hormones, sperm quality, and seminal antioxidant capacity in goat bucks under summer hot humid conditions". *Veterinary Research Communications*, *46*(4), pp. 1245-1257.

https://doi.org/10.1007/s11259-022-09991-8

Fouad, A. A.; Al-Sultan, A. I. and Yacoubi, M. T. (2011): "Coenzyme Q10 counteracts testicular injury induced by sodium arsenite in rats". *European Journal of Pharmacology*, 655(1-3), pp. 91-98. https://doi.org/10.1016/j.ejphar.2010.12.045

Gomez, S.D.; Bustos, P.S.; Sánchez, V.G., et al. (2020): "Trophoblast toxicity of the neonicotinoid insecticide acetamiprid and an acetamiprid-based formulation". *Toxicology*, 431, p.p. 152363.

https://doi.org/10.1016/j.tox.2020.152363.

Gu, Y.H.; Li, Y.; Huang, X.F.; et al. (2013): "Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development in vitro". *PloS one*, 8(7), e70112.

https://doi.org/10.1371/journal.pone.0070112

Guiekep, A.J.N.; Kenfack, A.; Ngoula, F., et al., (2019): "Attenuating effects of Mangifera indica leaves ethanolic extract against acetamiprid induced reproductive toxicity in male guinea pigs". *Veterinary Research Forum*, 10 (3), p.p.187–192.

https://doi: 10.30466/vrf.2019.9515.2292

Hassan, H.M.; Abdrazek, E.; Mahdi, M. R.; et al (2022): "Neuroprotective effect of naringin against cerebellar changes in Alzheimer's disease through modulation of autophagy, oxidative stress and tau expression: An experimental study". *Frontiers in Neuroanatomy***,** 97, pp. 1-17.

https://doi.org/10.3389/fnana.2022.1012422

Houchat, J.N.; Cartereau, A.; Le Mauff, A.; et al. (2020): "An overview on the effect of neonicotinoid insecticides on mammalian cholinergic functions through the activation of neuronal nicotinic acetylcholine receptor". *International Journal of Environmental Research and Public Health*, 17(9): 3222. *17*(9), p.p. 3222;
https://doi.org/10.3390/ijerph17093222

Humann-Guilleminot, S.; de Montaigu, C.T.; Sire, J.; et al., (2019): "A sublethal dose of the neonicotinoid insecticide acetamiprid reduces sperm density in a songbird". *Environmental Research*, *177*, pp. 108589.
https://doi.org/10.1016/j.envres.2019.108589

Ibrahim, H.Z.; El-Banna, S.G. and El Rafea, A.A. (2020): "Acetamiprid, insecticide -induced oxidative damage on reproductive parameters male rats". *Alexandria Journal of Veterinary Sciences*, 64(1) pp. 63-68.
https://doi: 10.5455/ajvs.78757.

Imamura, T.; Yanagawa, Y.; Nishikawa, K.; et al. (2010): "Two cases of acute poisoning with acetamiprid in humans". *Clinical Toxicology*, 48(8), pp. 851-853.
https://doi.org/10.3109/15563650.2010.517207

Karanth, S.; Yu, W.H.; Mastronardi, C.A.; et al. (2003): "Vitamin E stimulates luteinizing hormone -releasing hormone and ascorbic acid release from medial basal hypothalami of adult male rats". *Experimental Biology and Medicine*, *228*(7), pp. 779-785.
https://doi.org/10.1177/15353702-0322807-02

Kenfack, A.; Guiekep, N.A.J.; Ngoula, F.; et al (2018): "Reproductive toxicity of acetamiprid in male Guinea pig (Cavia porcellus)". *Journal of Veterinary Medicine and Animal Sciences*, *3*(4), pp. 105-111.
https://doi.org/10.31248/JASVM2018.101

Khafaji, S.S. (2023): "Antioxidant, anti-inflammatory, and anti-reprotoxic effects of kaempferol and vitamin E on lead acetate-induced testicular toxicity in male rats". *Open Veterinary Journal*, 13(12), pp. 1683–1695.
https://doi: 10.5455/OVJ.2023.v13.i12.17

Kong, D.; Zhang, J.; Hou, X.; et al. (2017): "Acetamiprid inhibits testosterone synthesis by affecting the mitochondrial function and cytoplasmic adenosine triphosphate production in rat Leydig cells". *Biology of Reproduction*, 96(1), pp. 254-265.
https://doi.org/10.1095/biolreprod.116.139550

Liao, S.; Omage, S.O.; Börmel, L.; et al. (2022): "Vitamin E and metabolic health: relevance of interactions with other micronutrients". *Antioxidants*, 11(9): pp. 1785.
https://doi.org/10.3390/antiox11091785

Marın, A.; Vidal, J.M.; Gonzalez, F. E.; et al. (2004): "Assessment of potential (inhalation and dermal) and actual exposure to acetamiprid by greenhouse applicators using liquid chromatography–tandem mass

spectrometry". *Journal of Chromatography B*, 804(2), pp. 269-275. https://doi.org/10.1016/j.jchromb.2004.01.022

Marzouki, S.; Dhouib, I. B.; Ben-abdessalem, C.; et al. (2017): "Specific immune responses in mice following subchronic exposure to acetamiprid". *Life Sciences*, 188, pp. 10-16.

https://doi.org/10.1016/j.lfs.2017.08.022

Mondal, S.; Mukhopadhayay, S.K.; Kumar, S.; et al (2015): "Haematobiochemical profile in chronic acetamiprid exposure in Sprague Dawley rats". *Advances in Animal and Veterinary Sciences*, 3(7), pp. 384-394.

http://dx.doi.org/10.14737/journal.aavs/2015/3.7.384.394

Mosbah, R.; Djerrou, Z. and Mantovani, A. (2018):" Protective effect of Nigella sativa oil against acetamiprid induced reproductive toxicity in male rats. *Drug and Chemical Toxicology*, 41(2), pp. 206 -212. https://doi.org/10.1080/01480545.2017.1337127

Najaran, H.; Rashtbari, H.; Mohammadi, A.; et al. (2019): "The protective effect of coenzyme Q10 and berberine on sperm parameters, with and without varicocel-ectomy in rats with surgically induced varicoceles". *Comparative Clinical Pathology*, 28, pp. 479-485.

https://doi.org/10.1007/s00580-018-2850-y

Neghab, M.; Momenbella-Fard, M.; Naziaghdam, R.; et al. (2014): "The effects of exposure to pesticides on the fecundity status of farm workers resident in a rural region of Fars province, southern Iran". *Asian Pacific Journal of Tropical Biomedicine*, 4(4), pp. 324-328.

https://doi.org/10.12980/APJTB.4.2014C586

Nishikimi, M.; Rao, N. A. and Yagi, K. (1972): "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen". *Biochemical and Biophysical Research Communications*, 46(2), pp. 849-854. https://doi.org/10.1016/s0006-291x(72)80218-3

Oda, S.S.; Waheeb, R.S. and El-Maddawy, Z.K. (2018): "Potential efficacy of Coenzyme Q10 against oxytetracycline-induced hepatorenal and reproductive toxicity in male rats". *Journal of Applied Pharmaceutical Science*, 8(1) pp. 098 -107.

https://dx.doi.org/10.7324/JAPS.2018.8115

Ognjanović, B.I.; Marković, S.D.; Đorđević, N.Z.; et al. (2010): "Cadmium-induced lipid peroxidation and changes in antioxidant defense system in the rat testes: Protective role of coenzyme Q10 and Vitamin E". *Reproductive Toxicology*, 29(2), pp. 191-197.

https://doi.org/10.1016/j.reprotox.2009.11.009

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): "Assay for lipid peroxides in

animal tissues by thiobarbituric acid reaction". *Analytical Biochemistry*, 95(2), pp. 351-358.

https://doi.org/10.1016/0003-2697(79)90738-3

Oyovwi, M.O.; Ben-Azu, B.; Agbonifo-Chijiokwu, E.; et al. (2022): "Possible mechanisms involved in the prevention and reversal of chlorpromazine-induced testicular damage by taurine and coenzyme-Q10 in rats". *Nutrire,* 47(2), pp. 31. https://doi.org/10.1186/s41110-022-00180-w

Öztaş, E.; Kara, M.; Boran, T.; et al. (2021): "Cellular stress pathways are linked to acetamiprid-induced apoptosis in SH-SY5Y neural cells". *Biology*, 10(9), pp. 820-837.

https://doi.org/10.3390/biology10090820

Pallotti, F.; Bergamini, C.; Lamperti, C.; et al. (2021): "The Roles of coenzyme Q in disease: direct and indirect involvement in cellular functions". *International Journal of Molecular Sciences*, 23(1), pp. 128. https://doi.org/10.3390/ijms23010128

Perrard, M. H.; Sereni, N.; Schluth-Bolard, C.; et al. (2016): "Complete human and rat ex vivo spermatogenesis from fresh or frozen testicular tissue". *Biology of Reproduction*, 95 (4), pp.1-10.

https://doi.org/10.1095/biolreprod.116.142802

Phogat, A.; Singh, J.; Kumar, V.; et al. (2022). Toxicity of the acetamiprid insecticide for mammals: A review. *Environmental Chemistry Letters*, 20, pp. 1453–1478. https://doi.org/10.1007/s10311-021-01353-1

Phogat, A.; Singh, J.; Kumar, V.; et al. (2023): "Berberine mitigates acetamiprid-induced hepatotoxicity and inflammation via regulating endogenous antioxidants and NF-κB/TNF-α signaling in rats". *Environmental Science and Pollution Research,* 30(37), pp. 87412-87423.

https://doi.org/10.1007/s11356-023-28279-1

Pirasath, S.; Senthuran, R.; Athirayan, C.; et al. (2021): "Acute poisoning with acetamiprid: A case report." *Journal of Medical Case Reports*, 15, pp. 1-5.

https://doi.org/10.1186/s13256-021-02919-x

Rachid, M.; Ibrahim, Y.M. and Aziez, C. (2013): "Nigella sativa oil protects against reproductive toxicity of acetamiprid insecticide in male rats". *Toxicology Letters*, 221, pp. 17-19.

http://dx.doi.org/10.1016%2Fj.toxle.2013.05.652

Ramadan, L.A.; Abd-Allah, A.R.; Aly, H.A.; et al (2002):" Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice". *Pharmacological Research*, 46(4), pp. 363-370.

https://doi.org/10.1016/S1043661802001718

Raouf, S. M. and Taha, A. T. (2021): "Effects of COQ10 with vitamin E supplementation on semen quality and seminal plasma parameters of

broiler breeder males". *Iraqi Journal of Veterinary Sciences*, 35(1), pp. 65-70. http://www.doi.org/10.33899/ijvs.2020.126287.1289

Renaud, M.; Akeju, T.; Natal-da-Luz, T.; et al. (2018): "Effects of the neonicotinoids acetamiprid and thiacloprid in their commercial formulations on soil fauna". *Chemosphere*, 194, pp. 85-93. https://doi.org/10.1016/j.chemosphere.2017.11.102

Salvio, G.; Cutini, M.; Ciarloni, A.; et al. (2021): "Coenzyme Q10 and male infertility: a systematic review". *Antioxidants*, 10(6) pp. 874.

https://doi.org/10.3390/antiox10060874

Selvestrel, G.; Lavado, G.J.; Toropova, A.P.; et al. (2022): "Monte Carlo models for sub-chronic repeated-dose toxicity: systemic and organ-specific toxicity". *International Journal of Molecular Sciences*, 23(12) pp. 6615.

https://doi.org/10.3390/ijms23126615

Sevim, Ç.; Akpınar, E.; Aksu, E.H.; et al., (2023): "Reproductive effects of s. boulardii on sub-chronic acetamiprid and imidacloprid toxicity in male rats". *Toxics.*, 11(2), pp 170-183.

https://doi.org/10.3390/toxics11020170

Shahin, M. (2018): "Hepatoprotective effect of ginseng, green tea, cinnamon their combination against aceta-miprid -induced oxidative stress in rats". *Asian Journal of Biology*, 5(1), pp. 1-13.

https://doi.org/10.9734/AJOB/2018/38202

Shakthi Devan, R.K.; Prabu, P.C. and Panchapakesan, S. (2015): "Immunotoxicity assessment of sub-chronic oral administration of acetamiprid in Wistar rats". *Drug and Chemical Toxicology*, *38*(3), pp. 328-336. https://doi.org/10.3109/01480545.2014.966382

Sharma, A.; Kshetriyanum, C.; Patel, B.; et al. (2021):" Prevention of arsenic induced testicular oxidative stress and DNA damage by coenzyme Q10 and vitamin E in Swiss albino mice". *Journal of Infertility and Reproductive Biology*, *9*(2), pp.93-99.

https://doi.org/10.47277/JIRB/9(2)/93

Shukla, S. and Dubey, K.K. (2018): "CoQ10 a super-vitamin: Review on application and biosynthesis". *3 Biotech*, 8(5), p.p. 249.

https://doi.org/10.1007/s13205-018-1271-6

Terayama, H.; Qu, N.; Endo, H.; et al. (2018): "Effect of acetamiprid on the immature murine testes". *International Journal of Environmental Health Research*, 28(6), pp.683-696.

https://doi.org/10.1080/09603123.2018.1504897

Toghan, R.; Amin, Y.A.; Ali, R.A.; et al. (2022): "Protective effects of folic acid against reproductive, hemato-logical, hepatic, and renal toxicity induced by Acetamiprid in male Albino rats". *Toxicology*, 469, 153115.

https://doi.org/10.1016/j.tox.2022.153115

Trošić, I.; Mataušić-Pišl, M.; Pavičić, I.; et al. (2013): "Histological and cytological examination of rat reproductive tissue after short-time intermittent radio-frequency exposure". *Archives of Industrial Hygiene and Toxicology*, 64(4), pp. 513-519. https://doi:10.2478/10004-1254-64-2013-2394

Yilmaz, B.O.; Korkut, A. and Erkan, M. (2018): "Sodium fluoride disrupts testosterone biosynthesis by affecting the steroidogenic pathway in TM3 Leydig cells". *Chemosphere*, 212, pp.447-455. https://doi.org/10.1016/j.chemosphere.2018.08.112

Yi-Wang, J.J.Z.; Xiang, H.Y.; Jia-Hua, Z., et al. (2012): "Acetamiprid residues in male mice and its effect on liver function". *Journal of Animal and Veterinary Advances*, 11(15), pp. 706 - 2710.

https://www.researchgate.net/profile/Jiao-Jiao-Zhang-2/publication/321824514

Yokoi, K.; Uthus, E.O. and Nielsen, F.H. (2003): "Nickel deficiency diminishes sperm quantity and movement in rats". *Biological Trace Element Research, 93*, pp.141-153.

https://doi.org/10.1385/BTER:93:1-3:141

Yousef, A.O.; Fahad, A.; Abdel Moneim, A.E.; et al. (2019):"The neuro-protective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities". *International Journal of Environmental Research and Public Health*, 16(16), pp. 2895.

https://doi.org/10.3390/ijerph16162895

Zayman, E., Gül, M., Erdemli, M.E., et al. (2022): "Biochemical and histo-pathological investigation of the protective effects of melatonin and vitamin E against the damage caused by acetamiprid in Balb-c mouse testicles at light and electron microscopic level". *Environmental Science and Pollution Research*, 29(31), pp.47571-47584.

https://doi.org/10.1007/s11356-022-19143-9

Zhang, J.J.; Yi, W.A.N.G.; Xiang, H.Y.; et al. (2011):" Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system". *Agricultural Sciences in China*, 10(5), pp. 786-796.

https://doi.org/10.1016/S1671-2927(11)60063-1

Zuščíková, L.; Bažány, D.; Greifová, H.; et al. (2023): "Screening of toxic effects of neonicotinoid insecticides with a focus on acetamiprid: A review". *Toxics*, 11(7) pp. 598.

https://doi.org/10.3390/toxics11070598

التأثيرات الوقائية لفيتامين ه وانزيم Q10 المساعد ضد السمية الإنـجابية الناجمة عن الأسيتامبريد في ذكور فئران سبراغ داولي البالغة.

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يعد الأسيتامبريد (ACE) أحد أكثر النيكوتينياميدات استخدامًا في جميع أنحاء العالم. تهدف الدراسة الحالية إلى دراسة التأثيرات الوقائية لفيتامين ه (Vit E) (100 وحدة دولية/كجم/يوم) و/أو الإنزيم المساعد (CoQ10) (75 مجم/كجم/يوم) ضد السمية التناسلية للذكور الناتجة من (ACE) (25 مجم/كجم/يوم). تم استخدام ثماني مجموعات من ذكور فئران سبراغ داولي البالغة (عدد كل منها= 6) على النحو التالي: المجموعة الضابطة، الزيت، (Vit E)، (CoQ10)، (ACE)، (ACE + Vit E)، (ACE + CoQ10)و (ACE+Vit E+CoQ10) .تلقت الفئران العلاج عن طريق الفم لمدة 60 يومًا. أظهر إعطاء (ACE) انخفاضاً ذو اهمية احصائية في كل من: (أوزان الجسم، أوزان الخصية، هرمون التستوستيرون في الدم، الهرمون اللوتيني في الدم، مضاد الأكسدة الجلوتاثيون فى الخصية، نشاط ديسموتاز فوق أكسيد فى الخصية ، عدد الحيوانات المنوية ونسبة حركة الحيوانات المنوية)؛ وذلك تماشياً مع زيادة ذات اهمية احصائية في ما يلي : (نسبة الحيوانات المنوية الميتة، نسبة الحيوانات المنوية غير الطبيعية، المالونديالدهيد في الخصية). اظهر الفحص النسجى لمجموعة (ACE) تغيرات تنكسية ملحوظة في الأنابيب المنوية مع زيادة تكون كل من (Caspase-3) وعامل نخر الورم ألفا فى الخصية واللذان تم الكشف عنهما باستخدام الكيمياء المناعية. أظهرت الاستخدام الاحادى لـ (Vit E) أو (CoQ10) مع (ACE) تحسنًا في التأثيرات النسيجية والأكسدة والالتهابية وموت الخلايا المبرمج وسمية السائل المنوي ، لكنها فشلت في تحسين هرمون التستوستيرون في الدم وأوزان الجسم والخصية. علاوة على ذلك، فإن التأثيرات الوقائية لاستخدام (Vit E) و (CoQ10) معا تفوقت بشكل كبيرعلى الاستخدام المنفصل لكلا العاملين في تحسين أوزان الجسم والخصية، وحركة الحيوانات المنوية، والحيوية، والتستوستيرون، والهرمون اللوتيني، وتكون Caspase-3. أخيرًا، استخدام كل من (Vit E) و(CoQ10)اظهر اثار وقائية ضد التسمم التناسلي الذكري الناجم عن(ACE) وان كان استخدام العاملين الوقئين معا كان أكثر فعالية.