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RESEARCH

Protective role of vitamin E against acrylamide-induced testicular toxicity from pregnancy to adulthood: insights into oxidative stress and aromatase regulation

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Abstract

Acrylamide (ACR) is a toxic chemical frequently encountered in daily life, posing health risks. This study aimed to elucidate the molecular-level mechanism of ACR's toxic effects on testicles and investigate whether Vitamin E can mitigate these effects. A total of 40 adult pregnant rats were utilized, divided into four groups: Control, ACR, Vitamin E, and ACR + Vitamin E. ACR and Vitamin E were administered to the mother rats during pregnancy and lactation, and to the male offspring until the 8th week post-birth. Serum hormone levels, oxidant-antioxidant parameters, histopathological examination of testicular tissue, and mRNA and protein levels of the testicular and liver aromatase gene were analyzed. Spermiogram analysis was conducted on the collected sperm samples from the male offspring. The results revealed that ACR exposure adversely affected hormone levels, oxidant-antioxidant parameters, histological findings, as well as aromatase gene and protein expressions. However, Vitamin E administration effectively prevented the toxic effects of ACR. These findings demonstrate that ACR application significantly impairs the reproductive performance of male offspring rats by increasing liver aromatase activity.

Keywords Acrylamide · Testicle · Spermiogram · Aromatase · Oxidative stress

Abbreviations

ACR	Acrylamide
Vit E	Vitamin E
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
RIPA	Radioimmunoprecipitation assay
SDS-PAGE	Sodium Dodecyl Sulfate–PolyAcrylamide Gel Electrophoresis
MDA	Malondialdehyde
SOD	Superoxide dismutase
CAT	Catalase
GSH-Px	Glutathione peroxidase
GSH	Glutathione
GST	Glutathione S-transferase
TAS	Total Antioxidant Status
TOS	Total Oxidant Status
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
ELISA	Enzyme-linked immunosorbent assay

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Introduction

Acrylamide (ACR) is widely used in medicine and industrial fields, such as paper, wastewater treatment, and textiles (Zhao et al. 2022). In 1994, the International Agency for Research on Cancer (IARC) classified ACR as a Group

2A carcinogen (Zhang and Zhang 2007). A significant milestone in ACR research was reached in 2002 when Swedish scientists discovered its presence in asparagine-rich foods after heat treatment (Capuano and Fogliano 2011). The processing of foods at temperatures above 120 °C leads to the formation of substantial amounts of ACR (Mottram et al. 2002). Studies have shown that exposure to ACR induces various toxic effects in the testicular tissues of rats, such as seminiferous tubule degeneration, increased apoptotic cell count, and varying degrees of spermatogonium degeneration (Khezerolou et al. 2018), (Hasanin et al. 2018). Additionally, ACR has been found to induce oxidative stress and tissue damage in the testicles, resulting in reduced levels of certain enzymes responsible for testosterone synthesis in Leydig cells and impairment of spermatogenesis (Abd-Elsalam et al. 2021).

Vitamin E, known for its anticarcinogenic and neuroprotective properties, is a crucial antioxidant that positively affects cell metabolism (Sharifi-Rad et al. 2020). Due to its lipophilic nature, Vitamin E easily crosses the placenta and exhibits strong protective effects by reducing inflammation and adhesion, particularly in tissues such as the brain, placenta, testicles, and others (Niki 2014), (Zaidi and Banu 2004). Furthermore, it enhances the immune system and improves metabolism as a free radical scavenger and antioxidant (Lewis et al. 2019). Studies have reported that Vitamin E supplementation supports the reproductive system by promoting testicular regeneration, as well as the production, maturation, and quality of spermatozoa (Peng et al. 2023) Moreover, supplementation with vitamin E significantly reduces ACR-induced oxidative tissue damage, aiming to protect against its toxic effects on testicular tissue (Hasanin et al. 2018).

The aromatase enzyme, responsible for irreversibly converting androgens to estrogens in steroidogenesis, plays a critical role in testicular development and associated diseases (Carreau et al. 2003). Testicular estrogens, binding to estrogen receptors (ERα and β), regulate sperm maturation in the efferent ducts and epididymis, thus preserving reproductive functions and regulating gonadal activity (Hess et al. 1997), (O'Donnell et al. 2001). Deficiency in aromatase and subsequent testicular estrogen deficiency have been linked to fertility issues, developmental challenges during puberty, decreased bone density, and disorders in bone development (Zirilli et al. 2008), (Grumbach 2011). Exposure to toxic compounds has been shown to affect aromatase levels and disrupt the androgen/estrogen balance, leading to disorders in testicular development and alterations in sperm morphology (Cassault-Meyer et al. 2014), (Park et al. 2020). Studies investigating the impact of ACR on testicular physiology and pathology have reported testicular tissue shrinkage, histopathological lesions, decreased sperm quality (Ma et al. 2011), and various disorders in the male reproductive system, including cryptorchidism (Souza et al. 2019).

Despite an increasing number of studies demonstrating the detrimental effects of ACR on testicular functions, its exact role in the development and physiology of the reproductive system remains incompletely understood. Therefore, the objective of this study was to investigate the potential link between testicular dysfunctions in male rat offspring exposed to ACR and their sperm functions, aromatase activity, and oxidative stress levels. Additionally, we examined the protective effect of vitamin E against potential damages and dysfunctions.

Materials and methods

Animals

Forty female Sprague Dawley rats weighing between 250–350 g were obtained from the Inönü University Experimental Animal Production and Research Center. The rats were individually housed overnight in single cages, with one male rat per female rat. Vaginal smears were collected from the female rats, and those showing the presence of sperm during microscopic examination were considered to be half a day pregnant. Subsequently, the pregnant rats were divided into cages based on the assigned treatment groups. After three weeks from the time the pregnant rats gave birth to their offspring, forty male rats were selected and assigned to cages according to the treatment groups. The study was continued with these male rats. Throughout the study, all rats had unrestricted access to normal pellet feed and water.

Experimental design

The study consisted of four groups: Control, Acrylamide (ACR), Vitamin E, and Acrylamide + Vitamin E (ACR + Vit E). Pregnant rats were assigned to cages based on their treatment groups, and the treatments began on the first day. The treatments were administered as follows: the control group received standard chow and drinking water, the ACR group received 100 ppm Acrylamide (Acrylamide; Sigma A8887) in their drinking water (Ogawa et al. 2012), (Takahashi et al. 2008; Takahashi et al. 2009), (Manjanatha et al. 2006), the Vitamin E group received 300 ppm α-Tocopherol acetate (DL-α-Tocopherol acetate, Water-Soluble Vitamin E, MP Biomedicals) in their drinking water (Tanaka et al. 1990), (Pool and Jaarsveld 1998), (Zingg 2018), and the ACR + Vit E group received 100 ppm Acrylamide and 300 ppm α-Tocopherol acetate in their drinking water. Pregnant rats gave birth to their offspring vaginally, and the offspring remained with their mothers for feeding until day 21. Maternal rats continued to receive ACR and/or Vit E during the

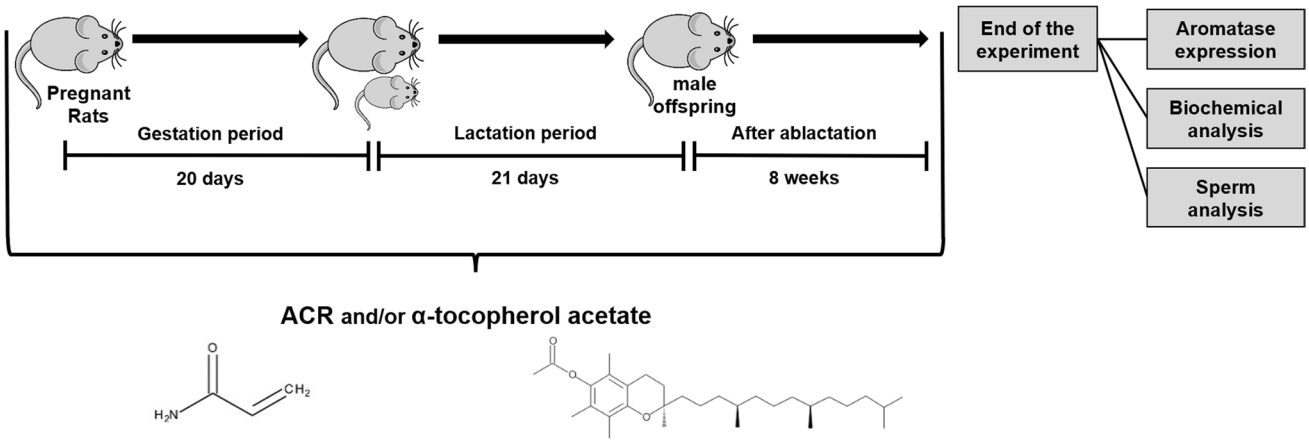

Fig. 1 Study design. The study was started with 40 pregnant rats. ACR and/or vitamin E were administered throughout the gestation period. After 20 days, rats delivered their fetuses by normal vaginal delivery. During the lactation period, ACR and/or Vit E continued to be administered to the dams in drinking water. At the end of the lactation period (at the end of 21 days), the male rats were randomly segregated into separate cages (10 male rats in each group) based on the groups. ACR and/or vitamin E administration was continued until the end of the study (at the end of 8 weeks)

lactation period. At the end of the third week, rats were placed in cages according to their treatment groups (10 male rats per group). Male rats continued to receive ACR and/or Vit E until the end of the eighth week, and the study concluded at the end of this period (Fig. 1). Since there was no significant change in the stability of both ACR (Adams et al. 2010) and Vitamin E (Cillard and Cillard 1980) aqueous solutions within three days, the water was changed three times a week (Monday, Wednesday, and Friday).

Sperm analysis

The sperm concentration in the right cauda epididymis was assessed using a modified approach described by Ciftci et al. (Ciftci et al. 2011), employing a hemocytometer. Sperm motility was measured using the freshly isolated right cauda epididymis, and a light microscope with a heated stage was utilized for this purpose (Ciftci et al. 2012). To evaluate the percentage of morphologically aberrant spermatozoa, the slides were stained with eosin-nigrosin (1.67 percent eosin, 10% nigrosin, and 0.1 M sodium citrate) and examined under a light microscope at 400 magnification. A total of 300 spermatozoa (2400 cells) were analyzed on each slide, and the head, tail, and overall abnormality rates of spermatozoa were calculated as percentages (Oguzturk et al. 2012).

RT-PCR analysis

Total RNA from testicular tissue was isolated using an RNA purification kit (RNeasy Mini Kit, QIAGEN). The cDNA synthesis was performed using the cDNA Synthesis Kit (Roche). RNA purity was assessed by measuring absorbance at 260 nm and 280 nm using a NanoDrop spectrophotometer (Epoch, Biotek, USA). The mRNA expression levels of Aromatase (Cyp19a1) and β-Actin (housekeeping gene) were analyzed using quantitative Real-Time PCR (Light Cycler, Roche) with the DNA Probes Master Kit (Roche), as well as gene-specific primers and probes (Table 1). For each sample, a master mix of 5 µl, 2 µl of PCR grade water, 7.5 µl of cDNA, and an appropriate volume of primers were prepared. The qRT-PCR procedure involved an initial denaturation at 95°C for 10 min, followed by 55 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 1 s. The relative quantification was calculated using the ΔΔCt formula and the cycle threshold (Ct) values.

Western blot analysis

Testicular tissues were homogenized in RIPA lysis buffer and incubated on ice for 20–30 min. After centrifugation at 20,000xg for 10 min at 4°C, the protein levels in the lysate

Table 1 Sequences of primer and probe numbers applied for qRT-PCR

Gene	Forward and Reverse sequence	Ref. Seq. Number
β-Actin	F: 5' CTGGCTCCTAGCACCATGA 3'	NM_031144
	R: 5'TAGAGCCACCAATCCACACA 3'	
Aromatase (Cyp19a1)	F:5'AACTACTACAATAAgATgTATggAgAgTTC3'	NM_017085
	R: 5' ATTCTCgTgCATgCCAATg 3'	

were determined. Subsequently, 25 µg of proteins were loaded onto a 10% SDS-PAGE gel and transferred to PVDF membranes. The membranes were blocked with 5% non-fat dry milk for 1 h at room temperature. Anti-Aromatase antibody (ab124776, Abcam) or β-actin (4970, CST) was incubated overnight at 4°C on the blocked membrane. Following this, the membranes were incubated with secondary antibodies (7074, CST) for 1 h, and the blots were visualized using ECL substrate (ECL Plus Substrate, Thermo Scientific). Band detection was carried out using the MicroChemi system (DNR Bio-Imaging System), and band densitometric analysis was performed using the ImageJ software.

Biochemical and hormonal analysis

For biochemical analysis (pH 7.4), tissues were homogenized in PBS. To measure the amount of Malondialdehyde (MDA), tissue homogenates were used directly. Subsequently, the homogenates were centrifuged at 5000 rpm (4 °C) for 30 min. After centrifugation, the clear supernatants were used for additional analyses and protein level determination. The levels of homogenized tissue MDA, indicating lipid peroxidation, were measured using the thiobarbituric acid reaction method described by Ohkawa et al. (Ohkawa et al. 1979). SOD (Superoxide dismutase) activity in testicle tissues was measured according to the method described by Sun et al. (Sun et al. 1988). The CAT (Catalase) activity in the supernatant was tested using a procedure adapted from Aebi's method (Aebi 1974). This method utilizes a spectrophotometer to monitor the rate of hydrogen peroxide disappearance over time at 240 nm. The GSH-Px (Glutathione peroxidase) activity was determined by measuring the oxidation of reduced nicotinamide-adenine dinucleotide phosphate at 340 nm, following the protocol described by Paglia and Valentin (Paglia and Valentine 1967). GSH (Glutathione) levels were determined using Ellman's method (Ellman 1959). The reduced GSH was treated with 5,5-dithiobis-2-nitrobenzoic acid and measured at 410 nm using spectrophotometry. The metabolite of nitric oxide, nitrite, was measured using the Greiss reagent, as previously reported (Ochoa et al. 1991). The activity of GST (Glutathione S-transferase) was calculated using the method described by Habig et al. (Habig et al. 1974). The concentrations of TAS (Total Antioxidant Status) and TOS (Total Oxidant Status) in the supernatant were determined using a spectrophotometer (microplate reader,Synergy H1) and commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey).

The levels of testosterone, estradiol, LH, and FSH in the blood were measured using ELISA kits (Bioassay Technology Laboratory, Shanghai, China). An ELISA microplate reader (BioTek Synergy H1) and a data analysis program (Gen5, BioTek Instruments) were employed for the measurements.

Histopathological examination

Testicle tissue specimens were dehydrated in an ethanol series, cleared in a xylene series, and embedded in paraffin wax for tissue processing. Paraffin-embedded specimens were sectioned into 6 µm thick slices, mounted on slides, and stained with hematoxylin–eosin (H-E). The sections were examined by a histologist using a light microscope (Nikon Eclipse Ni) and camera attachment (DS-Fi3). The images were analyzed with an Image Analysis System (Nikon NIS-Elements Documentation 5.02) (Nikon Corporation, Tokyo, Japan). To assess the degree of histopathological damage in the obtained sections, Johnson's grading system was used to score 100 randomly selected seminiferous tubules (Johnsen 1970).

Statistical analysis

The data was analyzed statistically using the SPSS 21.0 and Graphpad Prism 9 tools. To determine whether the data had a normal distribution, the Shapiro–Wilk test was employed. As the data did not follow a normal distribution, nonparametric statistical tests were applied, and the results were presented as median (min–max). The Kruskal–Wallis test was utilized to compare the groups, and pairwise comparisons were conducted using the Conover test. A significance level of $p < 0.05$ was considered statistically significant for all tests.

Results

Total body weights

Upon analyzing the results of total body weights, it was observed that administration of ACR led to a statistically significant reduction in body weights. In the ACR + Vit E group, the administration of Vitamin E resulted in a partial improvement in total body weights, which was also deemed

Table 2 Total body weights

Groups	Control	Vitamin E	ACR	ACR + Vit E
Total body weights (g)	336.7 ± 10.90^{a}	374.3 ± 29.11^{b}	218.6 ± 35.40^{c}	263.7 ± 12.38^{d}

The mean difference between groups with different superscript letters in the same column is statistically significant. (a,b, c and d; $p < 0{,}05$)

Table 3 Testicle, epididymis, seminal vesicle and total prostate weights

		Control	Vitamin E	ACR	ACR + Vit E
Testicle weights (g)	Left	1.340 ± 0.02^{a}	1.597 ± 0.04^{b}	1.028 ± 0.04^{c}	1.101 ± 0.01^{c}
	Right	1.384 ± 0.02^{a}	1.624 ± 0.05^{b}	1.088 ± 0.04^{c}	1.150 ± 0.01^{c}
Epididymis weights (g)	Left	0.634 ± 0.04^{a}	0.612 ± 0.01^{a}	0.467 ± 0.01^{b}	0.495 ± 0.02^{b}
	Right	0.642 ± 0.01^{a}	0.601 ± 0.03^{a}	0.488 ± 0.02^{b}	0.494 ± 0.03^{b}
Seminal vesicle weights (g)		1.162 ± 0.11^{a}	1.331 ± 0.10^{a}	1.067 ± 0.05^{a}	1.127 ± 0.08^{a}
Total Prostate weights (g)		0.240 ± 0.04^{a}	0.337 ± 0.04^{b}	0.327 ± 0.03^{b}	0.332 ± 0.04^{b}

The mean difference between groups with different superscript letters in the same column is statistically significant. (a,b and c; $p < 0,05$)

statistically significant. The specific weights of the live total body weights for each group are presented in Table 2.

Testicle, epididymis, seminal vesicle, and prostate weights

Upon analyzing the results of testicle, epididymis, seminal vesicle, and prostate weights, it was found that ACR administration significantly decreased the weights of the testicles and epididymis. However, the decrease in seminal vesicle weight was not statistically significant. On the other hand, ACR administration significantly increased the weight of the prostate tissue. In the ACR + Vit E group, the administration of Vitamin E partially improved the weights of the testicles, epididymis, seminal vesicle, and prostate. The weights of the reproductive system tissues for each group are presented in Table 3.

Spermiogram results

The examination of the Spermiogram results revealed that ACR administration significantly decreased the epididymal sperm concentration. Additionally, it led to a significant increase in the tail and head anomaly rate as well as the total abnormal sperm rate, while its impact on sperm motility was limited. Although the effects of Vitamin E administration on the decrease in sperm concentration in the ACR + Vit E group were limited, it reduced the abnormal sperm rate to the values observed in the control group (Table 4).

The qRT-PCR Results of Aromatase Gene mRNA

The analysis of aromatase gene mRNA in testicular tissue was performed using qRT-PCR, and the results indicated a significant increase in testicular aromatase/beta-actin mRNA ratios following ACR administration. These findings suggest that the administration of ACR upregulates the transcription of the testicular aromatase gene. In contrast, in the ACR + Vit E group, vitamin E administration resulted in a decrease in the aromatase/β-actin ratio, comparable to the control group values. The calculated testicular aromatase/β-actin mRNA ratios for the different groups are presented in Fig. 2.

To confirm the specificity of primer binding, β-actin and Aromatase cDNAs synthesized through RT-PCR were analyzed by electrophoresis on an agarose gel. The electrophoresis results revealed the formation of single DNA bands of the expected length for both β-actin and Aromatase genes (Fig. 2).

Western-blot results of testicular and liver aromatase protein

Western blot analysis was performed to assess the levels of Aromatase and β-actin proteins in testicular and liver tissues of the different groups. The analysis showed that ACR administration did not affect testicular aromatase levels, while it significantly increased liver aromatase levels. In the ACR + Vit E group, liver aromatase levels

Table 4 Spermiogram results

		Control	Vitamin E	ACR	ACR + Vit E
Sperm motility (%)		80.47 ± 1.88^{a}	83.56 ± 2.03^{a}	77.61 ± 1.19^{a}	80.71 ± 0.80^{a}
Epididymal sperm concentration (million/g tissue)		327.85 ± 2.59^{a}	$348.00. \pm 2,32^{a}$	242.28 ± 5.44^{b}	$263.28 \pm 4.56b^{b}$
Abnormal sperm rate (%)	Head	4.00 ± 0.30^{a}	3.00 ± 0.37^{b}	4.57 ± 0.36^{c}	3.85 ± 0.26^{a}
	Tail	4.00 ± 0.21^{a}	3.28 ± 0.20^{b}	5.28 ± 0.35^{c}	4.28 ± 0.28^{a}
	Total	8.00 ± 0.43^{a}	6.28 ± 0.64^{b}	9.85 ± 0.63^{c}	8.14 ± 0.34^{a}

The mean difference between groups with different superscript letters in the same column is statistically significant. (a,b and c; $p < 0,05$)

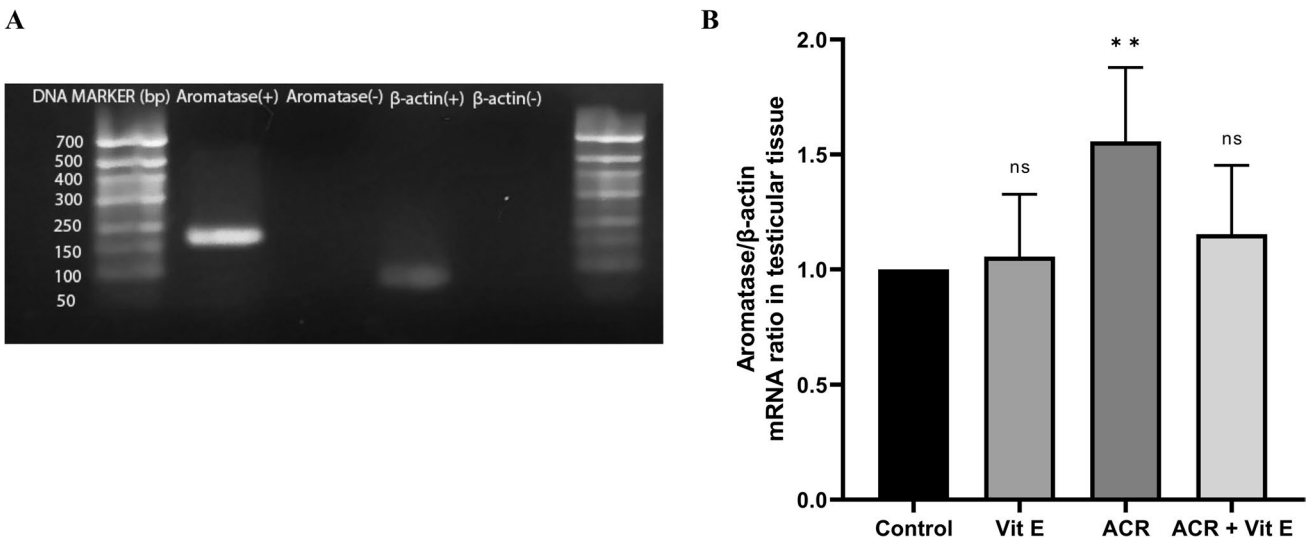

Fig. 2 Analysis of Aromatase mRNA gene expression in testicular tissue. **A.** Electropherogram of amplification of β-actin and Aromatase cDNA in RT-PCR. **A.** 50 bp DNA marker was used in electrophoresis (Bioron, 50 bp, catalog no: 304007). **B.** Aromatase gene expression in testicular tissue in all groups. Expression levels of the Aromatase gene were calculated by dividing the β-actin gene into all groups. Sample size is 6. All data were expressed as the mean ± SD (n = 6). Significantly different at $^{**}p < 0.01$ versus the control group

were significantly decreased. The protein bands and densitometric values for testicular and liver aromatase in the different groups are presented in Fig. 3.

Biochemical and hormonal analyzes

In the ACR group, compared to the control group, there was an observed increase in MDA ($p < 0.001$), NO ($p < 0.001$), and TOS ($p < 0.001$) levels, whereas GSH ($p < 0.01$), GSH-Px ($p < 0.001$), GST ($p < 0.001$), CAT ($p < 0.001$), SOD ($p < 0.01$), and TAS ($p < 0.01$) levels significantly decreased. Conversely, the groups receiving Vit E in conjunction with ACR showed increased levels of GSH, GSH-Px, GST, CAT, SOD, and TAS. Additionally, decreases were observed in the levels of MDA, NO, and TOS in the ACR + Vit E group compared to the ACR

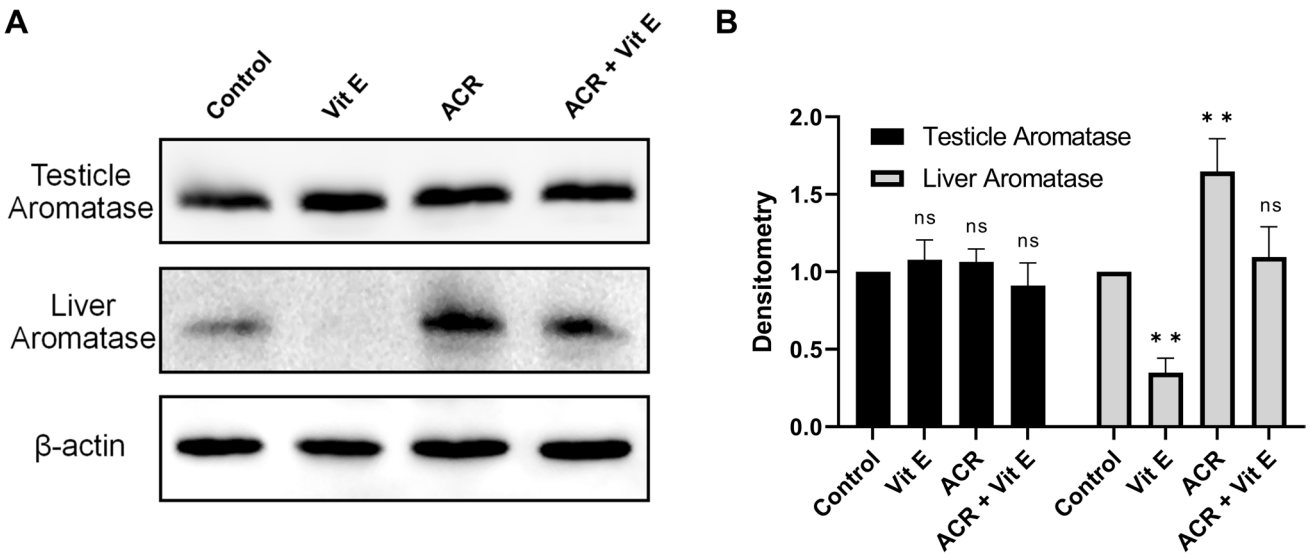

Fig. 3 Expression of Aromatase proteins in the testicle and liver was detected by Western blot assay. **A.** ACR application did not affect Aromatase protein expression in the testicle but increased Aromatase expression in the liver. **B.** The optical densities of the bands belonging to the test groups are shown in the histogram. All data were expressed as the mean ± SD (n = 3). Significantly different at $^{**}p < 0.01$ versus the control group

group (Fig. 4). The quantification of serum testosterone, estradiol, FSH, and LH levels in the groups revealed that ACR administration significantly decreased serum testosterone levels while significantly increasing serum estradiol levels. However, in the ACR + Vit E group, vitamin E administration brought the serum hormone levels back to those of the control group (Table 5).

Testicular histopathology results

The histological examination revealed normal seminiferous tubule and interstitial tissue areas in both the Control and Vit E groups. The germinative epithelium of the seminiferous tubules appeared multilayered, comprising cells from the spermatogenic series. Spermatozoa were observed in the lumens of certain seminiferous tubules (Fig. 5.AI, II). In the ACR group, varying degrees of damage were observed in the seminiferous tubules. Some seminiferous tubules exhibited complete germinative epithelial damage, desquamation of the germinative epithelium, and seminiferous tubule atrophy. The damage to the seminiferous tubules ranged from spermatogonia loss to milder spermatid and spermatozoon damage. Additionally, varying degrees of vacuolization and local degeneration were detected in Leydig cells within the

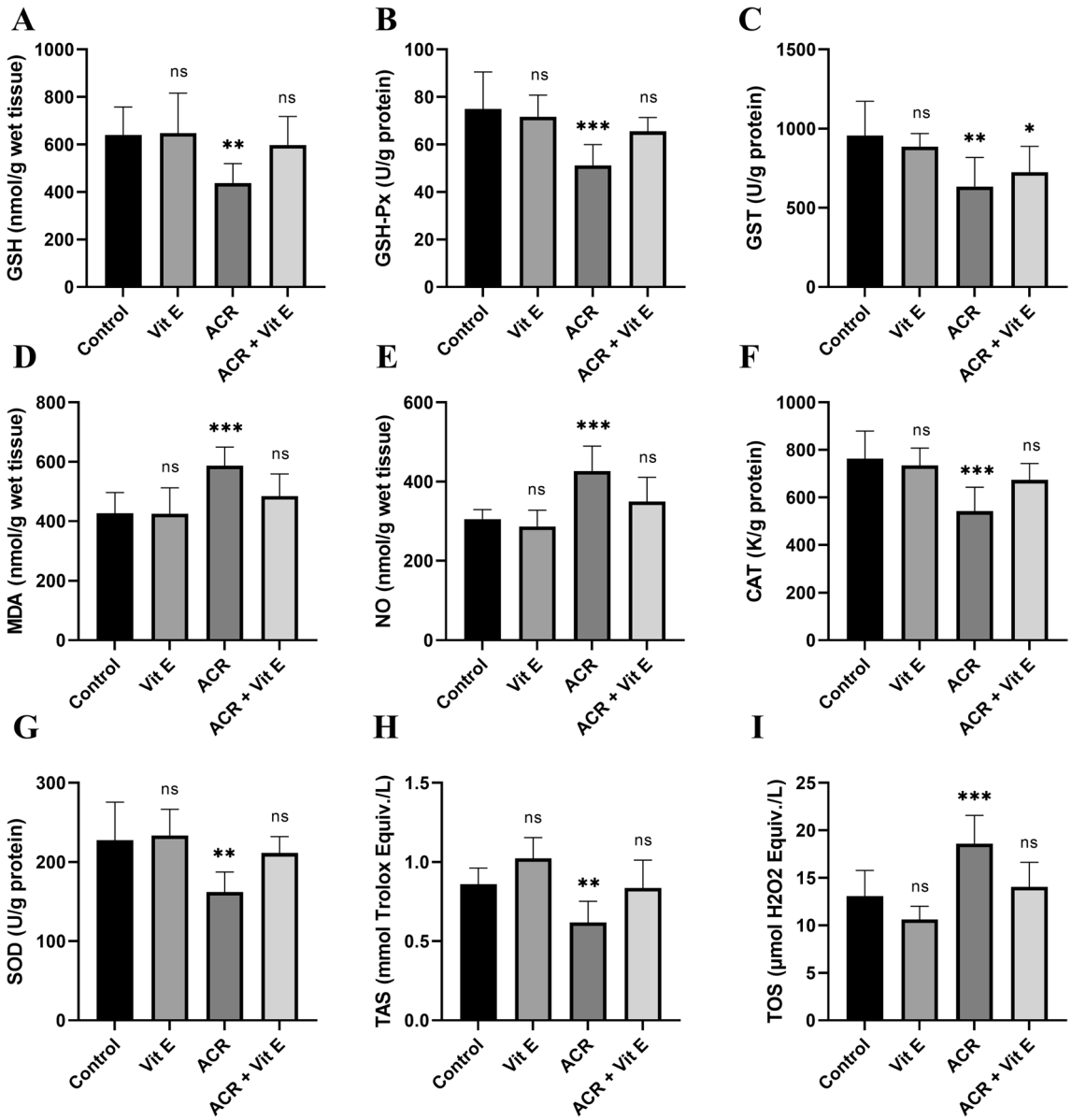

Fig. 4 Biochemical analysis results. Contents of GSH (**A**); activities of GSH-Px (**B**), activities of GST (**C**), contents of MDA (**D**), contents of NO (**E**), activities of CAT (**F**) activities of SOD (**G**); levels of TAS (**H**) and TOS (**I**) in the testicle of rat exposed to ACR and/or Vitamin E. All data were expressed as the mean ± SD. Significantly different at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ versus the control group

Table 5 Testosterone, Estradiol, FSH and LH results

Groups	Control	Vitamin E	ACR	ACR + Vit E	*p*
FSH (U/L)	7.76 (7.18–8.27)[a]	8.184 (7.44–8.86)[a]	6.99 (6.28–7.90)[a]	7.436 (6.68–8.33)[a]	> 0.05
LH (U/L)	5.92 (5.44–6.66)[a]	6.25 (5.60–6.60)[a]	5.47 (5.12–6.10)[a]	5.83 (5.22–6.29)[a]	> 0.05
Testosterone (ng/mL)	19.46 (18.19–22.03)[a]	20.94 (19.78–22.38)[a]	14.03 (12.48–16.14)[b]	18.53 (17.75–19.66)[a]	< 0.05
Estradiol (ng/L)	607.24 (588.99–617.51)[a]	603.25 (588.76–612.70)[a]	707.85 (697.04–720.92)[b]	627.33 (599.41–638.30)[a]	< 0.05

The mean difference between groups with different superscript letters in the same column is statistically significant. (a,b and c; $p < 0{,}05$)

interstitial spaces **(**Fig. 5**.** A.-III**).** In the ACR + Vit E group, shedding of the germinative epithelium and enlargement of the intercellular spaces were observed in certain seminiferous tubules. The Leydig cells in the interstitial spaces exhibited normal histomorphology. The level of histological damage in this group was higher than that of the control and Vit E groups but lower than that of the ACR group **(**Fig. 5**.** A-IV**).** According to the Johnsen Score evaluation, the score

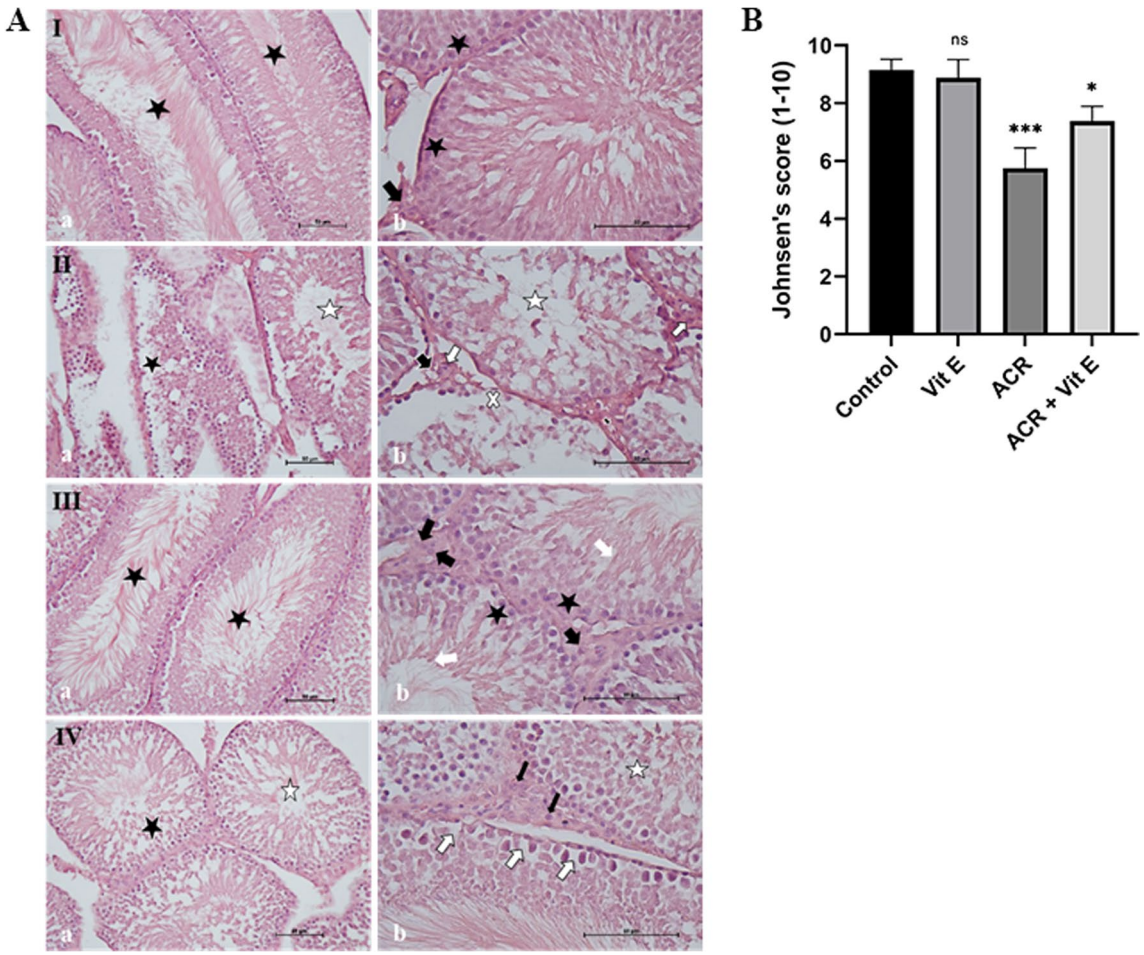

Fig. 5 Histopathological findings of testicular tissue in all groups. **A. I**: Control group: a) Normal histological appearance (star). HE, × 20. b) Normal seminiferous epithelium (star) and interstitial tissue (Arrow) in seminiferous tubules. HE, × 40. **A.II**: ACR group: a) Degeneration (black star) and partial damage (white star) in the seminiferous epithelium in the seminiferous tubules. HE, × 20. b) Damage to the seminiferous tubules, loss of spermatids (white star), loss of spermatogonia in the seminiferous tubule epithelium (X), varying degrees of damage to the interstitial spaces and Leydig cells (white arrow), vascular damage (black arrow). HE, × 40. **A.III**: Vit E group: a) Normal histological appearance (star). HE, × 20. b) Interstitial tissue and Leydig cells (black arrow) in normal appearance, the normal seminiferous epithelium (star), spermatids, and spermatozoa (white arrow) in seminiferous tubules. HE, × 40. **A.IV**: ACR + Vit E group: a) Seminiferous tubules with normal histological appearance (white star) and seminiferous tubule epithelium (black arrow). HE, × 20. b) Germinative epithelium shedding in the normal seminiferous epithelium (white star) and some seminiferous tubules, enlargement of intercellular spaces (white arrow), interstitial areas, and Leydig cells in normal histological appearance. HE, × 40. **B.** Johnsen's testicular score in different groups. All data were expressed as the mean ± SD. Significantly different at * $p < 0.05$ and *** $p < 0.001$ versus the control group

obtained in the ACR + Vit E group was higher than that of the ACR group, and these values were quite close to those of the control and Vit E groups (Fig. 5. B).

Discussion

Embryogenesis is the most crucial process through which toxicants crossing the blood-tissue barrier can impact fetal development. The fetal and neonatal periods, when major organs undergo formation and functional development, represent the most vulnerable stage for organ deformities caused by toxicants. Therefore, our study aimed to assess the effects of exposure to ACR from the fetal to postnatal period on testicular function. We examined the mechanisms underlying ACR-induced toxicity, with a specific focus on sperm function, aromatase activation, and oxidant damage. Additionally, we investigated the potential protective role of vitamin E. Our results demonstrate that ACR administration reduced sperm quality, increased aromatase gene expression, and significantly induced oxidative damage. Conversely, vitamin E administration ameliorated testicular damage induced by ACR exposure by modulating sperm functions, aromatase activity, and restoring redox balance.

ACR is a toxic chemical that is not naturally present in foods but is formed as a byproduct of heat treatment. Studies have reported that ACR can be generated during the preparation of carbohydrate- and protein-rich foods at temperatures exceeding 120 °C. Consequently, the consumption of these foods can lead to the accumulation of toxic levels of ACR in various tissues (World Health Organization 2003). ACR-induced tissue toxicity has been reported to have a particularly detrimental effect on the brain, liver, heart, immune system, and reproductive system, causing dysfunction in these tissues depending on the level of exposure (Kunnel et al. 2019). Studies investigating the toxic effects of ACR on the reproductive system have demonstrated that ACR influences reproductive hormones, resulting in decreased testosterone and progesterone levels, as well as increased estradiol levels (Yassa et al. 2014), (Lebda et al. 2014). Another study involving rats exposed to ACR showed a decrease in testosterone accompanied by a decrease in sperm concentration (Wang et al. 2010). When analyzing the hormone levels quantified in our study, we observed that ACR administration significantly decreases serum testosterone levels and significantly increases serum estradiol levels, while the hormone levels in the ACR + Vit E group return to the values of the control group. This may be attributed to the damage caused by ACR to Leydig cells, which accelerates the conversion of testosterone to estradiol by increasing aromatase activity in the liver. The normalization of hormone levels in the ACR + Vit E group may be due to vitamin E's protective effect on Leydig cells and its suppression of the increase in aromatase protein levels in the liver.

Regarding the effects of ACR exposure on body and testicular weights, previous studies have reported that ACR administration at different doses reduced both body and testicular weights in rats (Wang et al. 2010). In our study, we examined the weight measurements of reproductive tissues and found that ACR administration significantly reduced testicular and epididymis weights. However, when vitamin E was administered, it partially prevented this negative effect. The reduction in reproductive tissue weights caused by ACR may be attributed to its damaging effect on cell structure, inhibition of cell proliferation and regeneration, and overall suppression of protein synthesis in these tissues.

Another interesting finding of our study was the decrease in the rate of abnormal sperm and the increase in testicular and prostate weights in the vitamin E group compared to the control group. Vitamin E, with its antioxidant properties (Fiedor and Burda 2014), contributes to organ development by preventing cell damage. It also plays a crucial role in maintaining the stability of cell membranes (Rizvi et al. 2014), (Traber 2008), which enables healthy cell division, proliferation, and DNA repair. These properties make it important for metabolic processes related to the male reproductive system. Previous studies have shown that vitamin E supplementation increased testicular sperm count and motility (Anvari et al. 2020), as well as testicular weight at different doses (Hong et al. 2009). Other studies have reported that vitamin E enhanced sperm motility, viability, and concentration, while reducing the percentage of major sperm defects (Hatamoto et al. 2006), (Safa et al. 2016), (Ozer Kaya et al. 2020). Furthermore, decreased levels of enzymatic and non-enzymatic antioxidants in Leydig cells have been associated with impaired spermatogenesis and decreased epididymal sperm count (Cao et al. 2004). Therefore, increasing cellular antioxidant levels with vitamin E may effectively reduce abnormal sperm count. Our study demonstrates that the observed decrease in abnormal sperm rate, particularly in terms of sperm morphology, in the vitamin E group suggests that vitamin E may reduce the rate of abnormal sperm by supporting the antioxidant defense system in sperm cells.

Studies investigating the effects of ACR on spermatogenesis have observed decreases in sperm reserves and abnormal sperm in groups treated with ACR. Additionally, degeneration of germinal cells, atrophied Leydig cells, and a decrease in spermatozoa in the lumen have been reported (Nesreen et al. 2011). Atrophy of seminiferous tubules, presence of giant cells, deformation of connective tissue, and a decrease in Leydig, Sertoli, and spermatogenic cells were also observed in rat offspring exposed to ACR (Şen et al. 2015), (Kermani-Alghoraishi et al. 2010). Consistent with the literature, our study showed a significant increase in the rate of total abnormal sperm, sperm with head and tail anomalies, and a decrease in sperm motility and epididymal

sperm concentration in the ACR group. Furthermore, we observed a reduction in seminiferous tubule diameters and the formation of numerous giant cells in the seminiferous epithelium. However, the administration of vitamin E reversed these anomalies to the values observed in the control group. These findings suggest that the considerable improvement in abnormal sperm ratio due to vitamin E may be attributed to the significant reduction in histopathological damage caused by ACR in testicular tissue, as well as the restoration of spermatogonia and the structural integrity of spermatids and spermatozoa.

The complete relationship between the aromatase enzyme, which plays a crucial role in reproductive functions, and ACR-induced toxicity in testicular and liver tissues has not yet been fully understood. Our study aimed to investigate the levels of aromatase gene and protein in the testicles and livers of rats treated with ACR. In this context, we observed that ACR administration significantly increased the mRNA levels of the aromatase gene in the testicles. However, it did not have an effect on the conversion of mRNA to aromatase protein. Conversely, ACR notably increased the expression of aromatase protein in the liver. Interestingly, when vitamin E was administered to the ACR + Vitamin E group, it reduced the levels of aromatase protein in the liver to values comparable to those of the control group. Thus, vitamin E exhibited a significant reduction in aromatase protein levels in the liver.

Several studies have assessed the relationship between testicular damage and factors such as aromatase levels, oxidative stress, and reproductive hormones to understand the molecular-level changes induced by modulators that affect testicular functions. In a study conducted on male rats, it was reported that functional disorders in the hypothalamus-pituitary axis led to the overactivation of the aromatase enzyme. This overactivation resulted in decreased testosterone levels and increased reactive oxygen species (ROS), which were identified as key contributors to testicular damage (Li et al. 2023). Another study emphasized the importance of antioxidants in the spermatogenesis process. Antioxidants were found to prevent testicular damage by increasing the levels of testicular reduced glutathione (GSH), reducing nitric oxide and malondialdehyde levels, and modulating aromatase levels (Atta et al. 2017).

ACR disrupts cellular redox homeostasis and leads to an excessive increase in ROS (Orta-Yilmaz 2020), (Maan et al. 2020). ACR-induced oxidative stress-related toxicity has been reported to cause lipid peroxidation (Zamani et al. 2017). On the other hand, GSH serves as a natural antioxidant and plays a pivotal role in cellular defense mechanisms (Yonar 2013). Both in vitro and in vivo studies have demonstrated that ACR induces oxidative stress by elevating ROS levels, including peroxides, superoxides, and hydroxyl radicals, while reducing GSH levels (Elhelaly et al. 2019). In addition to its role as a non-enzymatic scavenger of oxygen radicals, GSH also acts as a coenzyme for GSH-Px (Tsukamoto et al. 2002). As a consequence of ROS elimination, GSH is considerably depleted, leading to a decline in its levels. Another important player in the neutralization of free radicals is GST (Yonar 2013). The reduction in GSH content results in decreased activity of the GST enzyme, which is dependent on GSH concentration. Increased levels of nitric oxide (NO) also trigger the oxidation of proteins, lipids, and DNA, disrupting metabolic pathways and cellular functions, ultimately leading to cell death (Tse 2017), (Wink et al. 2011). Our findings are consistent with previous research in the literature (Abd-Elsalam et al. 2021), (Abdel-Daim et al. 2014). We observed a decrease in CAT, GSH-Px, and GST activities, as well as TAS levels in the ACR group, while noting a significant increase in MDA and TOS levels. Moreover, our study revealed a parallel increase in NO levels along with the rise in oxidation products and the decline in antioxidant enzymes. We detected a significant decrease in the activity of SOD (Zhang et al. 2010), one of the primary antioxidant enzymes in semen. Depletion of GSH and SOD to critical levels may elevate lipid peroxidation, resulting in elevated concentrations of ROS in the testicles. Antioxidants serve as the primary defense against oxidative stress induced by free radicals (Agarwal et al. 2005). Previous reports have demonstrated that Vitamin E, a potent antioxidant, exerts a protective effect against ACR-induced ROS production in various tissues (Rajeh and Khayyat 2017). Vitamin E is a crucial antioxidant component of spermatozoa and safeguards the cell membranes against ROS (Yousef et al. 2003), thus representing a promising prophylactic agent against ACR-induced toxicity (Nesreen et al. 2011). Consequently, the increased production of ACR-induced free radicals in the testicles might have been counteracted by Vitamin E. Vitamin E functions as a powerful chain-breaking antioxidant (Hasanin et al. 2018). Our findings indicate that this detrimental effect is largely mitigated and restored to values similar to those observed in the control group.

Conclusion

In previous studies, it has been reported that administration of ACR leads to weight loss in reproductive tissues such as the testicles and epididymis. This weight loss has been associated with histopathological damage in the testicular tissue, oxidative stress, and adverse effects on sperm concentration and reproductive hormones. Our findings align with these previous studies, providing further evidence of the detrimental effects of ACR on the reproductive system. Importantly, this study is the first to demonstrate the damage to the male offspring's reproductive system following ACR administration during

pregnancy and lactation. Additionally, we have made a novel observation in this study, showing that ACR administration significantly increases aromatase protein levels in the liver. This increase in liver aromatase activity leads to a decrease in serum testosterone levels, as testosterone is converted to estradiol at a higher rate. Based on these findings, we propose that the reprotoxic effects of ACR on the male rats' reproductive system are likely due to the significant increase in liver aromatase activity and subsequent reduction in serum testosterone levels in male offspring rats. However, further studies examining other tissues, such as adipose and muscle tissue, which are known to express aromatase, are required to fully validate the proposed mechanism of reproductive toxicity caused by ACR.

Author Contributions MMÜ conducted the experiments and data analysis and wrote the paper; SG conducted the experiments; NÜ conducted the research and wrote the paper; TŞ, and MA carried out the spermiogram analysis; YÇ carried out the gene expression analysis; MG, and EZ carried out the histopathologic analysis; YT conceived the study and designed experiments. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest The authors declare no conflict of interest, financial or otherwise.

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References

Abdel-Daim MM, AbdEldaim MA, Hassan AGA (2014) Trigonella foenum-graecum ameliorates acrylamide-induced toxicity in rats: Roles of oxidative stress, proinflammatory cytokines, and DNA damage. Biochem Cell Biol 93:192–8. https://doi.org/10.1139/BCB-2014-0122

Abd-Elsalam RM, El Badawy SA, Ogaly HA, Ibrahim FM, Farag OM, Ahmed KA (2021) Eruca sativa seed extract modulates oxidative stress and apoptosis and up-regulates the expression of Bcl-2 and Bax genes in acrylamide-induced testicular dysfunction in rats. Environ Sci Pollut Res 28:53249–53266. https://doi.org/10.1007/s11356-021-14532-y

Adams A, Hamdani S, Van LF, Méjri S, De Kimpe N (2010) Stability of acrylamide in model systems and its reactivity with selected nucleophiles. Food Res Int 43:1517–1522. https://doi.org/10.1016/J.FOODRES.2010.04.033

Aebi H (1974) Methods of Enzymatic Analysis. Catalase 673–84. https://doi.org/10.1016/B978-0-12-091302-2.50032-3

Agarwal A, Prabakaran SA, Said TM (2005) Prevention of oxidative stress injury to sperm. J Androl 26:654–660. https://doi.org/10.2164/JANDROL.05016

Anvari M, Talebi AR, Mangoli E, Shahedi A, Ghasemi MR, Pourentezari M (2020) Effects of acrylamide in the presence of vitamin E on sperm parameters, chromatin quality, and testosterone levels in mice. Clin Exp Reprod Med 47:101. https://doi.org/10.5653/CERM.2019.03230

Atta MS, Almadaly EA, El-Far AH, Saleh RM, Assar DH, Al Jaouni SK et al (2017) Thymoquinone Defeats Diabetes-Induced Testicular Damage in Rats Targeting Antioxidant, Inflammatory and Aromatase Expression. Int J Mol Sci 18:919. https://doi.org/10.3390/IJMS18050919

Cao L, Leers-Sucheta S, Azhar S (2004) Aging alters the functional expression of enzymatic and non-enzymatic anti-oxidant defense systems in testicular rat Leydig cells. J Steroid Biochem Mol Biol 88:61–67. https://doi.org/10.1016/j.jsbmb.2003.10.007

Capuano E, Fogliano V (2011) Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT Food Sci Technol 44:793–810. https://doi.org/10.1016/J.LWT.2010.11.002

Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B, Bourguiba S (2003) Aromatase expression and role of estrogens in male gonad : a review. Reprod Biol Endocrinol 1:35. https://doi.org/10.1186/1477-7827-1-35

Cassault-Meyer E, Gress S, Séralini GÉ, Galeraud-Denis I (2014) An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality. Environ Toxicol Pharmacol 38:131–140. https://doi.org/10.1016/J.ETAP.2014.05.007

Ciftci O, Beytur A, Cakir O, Gurbuz N, Vardi N (2011) Comparison of reproductive toxicity caused by cisplatin and novel platinum-N-heterocyclic carbene complex in male rats. Basic Clin Pharmacol Toxicol 109:328–333. https://doi.org/10.1111/J.1742-7843.2011.00737.X

Ciftci O, Ozdemir I, Aydin M, Beytur A (2012) Beneficial effects of chrysin on the reproductive system of adult male rats. Andrologia 44:181–186. https://doi.org/10.1111/J.1439-0272.2010.01127.X

Cillard J, Cillard P (1980) Behavior of alpha, gamma, and delta tocopherols with linoleic acid in aqueous media. J Am Oil Chem Soc 57:39–42. https://doi.org/10.1007/BF02675523/METRICS

de Souza NP, Cardoso AF, Gomide LMM, Lima TRR, Miot HA, Martino-Andrade AJ et al (2019) Experimental cryptorchidism enhances testicular susceptibility to dibutyl phthalate or acrylamide in Sprague-Dawley rats. Hum Exp Toxicol 38:899–913. https://doi.org/10.1177/0960327119845040

Elhelaly AE, AlBasher G, Alfarraj S, Almeer R, Bahbah EI, Fouda MMA et al (2019) Protective effects of hesperidin and diosmin against acrylamide-induced liver, kidney, and brain oxidative damage in rats. Environ Sci Pollut Res Int 26:35151–35162. https://doi.org/10.1007/S11356-019-06660-3

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77. https://doi.org/10.1016/0003-9861(59)90090-6

Fiedor J, Burda K (2014) Potential Role of Carotenoids as Antioxidants in Human Health and Disease. Nutrients 6:466. https://doi.org/10.3390/NU6020466

Grumbach MM (2011) Aromatase deficiency and its consequences. Adv Exp Med Biol 707:19–22. https://doi.org/10.1007/978-1-4419-8002-1_5/COVER

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-Transferases: The First Enzymatic Step in Mercapturic Acid Formation. J Biol Chem 249:7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Hasanin N, Sayed N, Ghoneim F, Al-Sherief S (2018) Histological and Ultrastructure Study of the Testes of Acrylamide Exposed Adult Male Albino Rat and Evaluation of the Possible Protective Effect of Vitamin E Intake. J Microsc Ultrastruct 6:23. https://doi.org/10.4103/JMAU.JMAU_7_18

Hatamoto LK, Baptista Sobrinho CA, Nichi M, Barnabe VH, Barnabe RC, Cortada CNM (2006) Effects of dexamethasone treatment (to mimic stress) and Vitamin E oral supplementation on the spermiogram and on seminal plasma spontaneous lipid peroxidation and antioxidant enzyme activities in dogs. Theriogenology 66:1610–1614. https://doi.org/10.1016/J.THERIOGENOLOGY.2006.02.012

Hess RA, Gist DH, Bunick D, Lubahn DB, Farrell A, Bahr J et al (1997) Estrogen Receptor (α and β) Expression in the Excurrent Ducts of the Adult Male Rat Reproductive Tract. J Androl 18:602–611. https://doi.org/10.1002/J.1939-4640.1997.TB02437.X

Hong Z, Hailing L, Hui M, Guijie Z (2009) Effect of vitamin E supplementation on development of reproductive organs in Boer goat. Anim Reprod Sci 113:93–101. https://doi.org/10.1016/J.ANIREPROSCI.2008.05.076

Johnsen SG (1970) Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1:2–25. https://doi.org/10.1159/000178170

Kermani-Alghoraishi M, Anvari M, Talebi AR, Amini-Rad O, Ghahramani R, Miresmaili SM (2010) The effects of acrylamide on sperm parameters and membrane integrity of epididymal spermatozoa in mice. Eur J Obstet Gynecol Reprod Biol 153:52–55. https://doi.org/10.1016/J.EJOGRB.2010.07.008

Khezerolou A, Alizadeh-Sani M, Zolfaghari Firouzsalari N, Ehsani A (2018) Formation, Properties, and Reduction Methods of Acrylamide in Foods: A Review Study. J Nutr Fast Health 6:52–9. https://doi.org/10.22038/JNFH.2018.34179.1133

Kunnel SG, Subramanya S, Satapathy P, Sahoo I, Zameer F (2019) Acrylamide Induced Toxicity and the Propensity of Phytochemicals in Amelioration: A Review. Cent Nerv Syst Agents Med Chem 19:100–113. https://doi.org/10.2174/1871524919666190207160236

Lebda M, Gad S, Gaafar H (2014) Effects of Lipoic Acid on Acrylamide Induced Testicular Damage. Mater Sociomed 26:208. https://doi.org/10.5455/MSM.2014.26.208-212

Lewis ED, Meydani SN, Wu D (2019) Regulatory role of vitamin E in the immune system and inflammation. IUBMB Life 71:487–494. https://doi.org/10.1002/IUB.1976

Li B, Li J, Akbari A, Baziyar P, Hu S (2023) Evaluation of Expression of Cytochrome P450 Aromatase and Inflammatory, Oxidative, and Apoptotic Markers in Testicular Tissue of Obese Rats (Pre) Treated with Garlic Powder. Evid Based Complement Alternat Med 2023. https://doi.org/10.1155/2023/4858274

Ma Y, Shi J, Zheng M, Liu J, Tian S, He X et al (2011) Toxicological effects of acrylamide on the reproductive system of weaning male rats. Toxicol Ind Health 27:617–627. https://doi.org/10.1177/0748233710394235

Maan AA, Anjum MA, Khan MKI, Nazir A, Saeed F, Afzaal M et al (2020) Acrylamide Formation and Different Mitigation Strategies during Food Processing – A Review. Food Rev Intl 38:70–87. https://doi.org/10.1080/87559129.2020.1719505

Manjanatha MG, Aidoo A, Shelton SD, Bishop ME, McDaniel LP, Lyn-Cook LE et al (2006) Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. Environ Mol Mutagen 47:6–17. https://doi.org/10.1002/EM.20157

Mottram DS, Wedzicha BL, Dodson AT (2002) Acrylamide is formed in the Maillard reaction. Nature 419:448–449. https://doi.org/10.1038/419448a

Nesreen R, SamarAl S, Nasra A, Sufian EA (2011) Characterization of acrylamide mediated testicular toxicity in rat: light and electron microscopic study. Kuwait Med J 43:196–205

Niki E (2014) Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radic Biol Med 66:3–12. https://doi.org/10.1016/J.FREERADBIOMED.2013.03.022

O'Donnell L, Robertson KM, Jones ME, Simpson ER (2001) Estrogen and spermatogenesis. Endocr Rev 22:289–318. https://doi.org/10.1210/EDRV.22.3.0431

Ochoa JB, Udekwu AO, Billiar TR, Curran RD, Cerra FB, Simmons RL et al (1991) Nitrogen oxide levels in patients after trauma and during sepsis. Ann Surg 214:621–626. https://doi.org/10.1097/00000658-199111000-00013

Ogawa B, Wang L, Ohishi T, Taniai E, Akane H, Suzuki K et al (2012) Reversible aberration of neurogenesis targeting late-stage progenitor cells in the hippocampal dentate gyrus of rat offspring after maternal exposure to acrylamide. Arch Toxicol 86:779–790. https://doi.org/10.1007/S00204-012-0801-Y

Oguzturk H, Ciftci O, Aydin M, Timurkaan N, Beytur A, Yilmaz F (2012) Ameliorative effects of curcumin against acute cadmium toxicity on male reproductive system in rats. Andrologia 44:243–249. https://doi.org/10.1111/J.1439-0272.2012.01273.X

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Orta-Yilmaz B (2020) Protective effects of vitamin C and curcumin against acrylamide toxicity in embryonic fibroblast cells. Toxicol Environ Chem 101:389–403. https://doi.org/10.1080/02772248.2020.1711910

Ozer Kaya S, Gur S, Erisir M, Kandemir FM, Benzer F, Kaya E et al (2020) Influence of vitamin E and vitamin E-selenium combination on arginase activity, nitric oxide level and some spermatological properties in ram semen. Reprod Domest Anim 55:162–169. https://doi.org/10.1111/RDA.13601

Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158–169. https://doi.org/10.5555/URI:PII:0022214367900765

Park HJ, Zhang M, Lee WY, Hong KH, Do JT, Park C, et al. (2020) Toxic Effects of Nonylphenol on Neonatal Testicular Development in Mouse Organ Culture. Int J Mol Sci 21. https://doi.org/10.3390/IJMS21103491

Peng M, Wu J, Wang W, Liao T, Xu S, Xiao D, et al. (2023) Alpha-tocopherol enhances spermatogonial stem cell proliferation and restores mouse spermatogenesis by up-regulating BMI1. Front Nutr 10. https://doi.org/10.3389/FNUT.2023.1141964

Pool GF, Jaarsveld HV (1998) Dietary iron elevates LDL-cholesterol and decreases plasma antioxidant levels: influence of antioxidants - PubMed. Res Commun Mol Pathol Pharmacol 100:139–150

Rajeh NA, Khayyat D (2017) Effect of the combined administration of vitamin-E and 5-aminosalicylic acid on acrylamide-induced testicular toxicity. J Taibah Univ Med Sci 12:445–454. https://doi.org/10.1016/J.JTUMED.2017.03.005

Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F (2014) The Role of Vitamin E in Human Health and Some Diseases. Sultan Qaboos Univ Med J 14:e157

Safa S, Moghaddam G, Jozani RJ, Daghigh Kia H, Janmohammadi H (2016) Effect of vitamin E and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen. Anim Reprod Sci 174:100–106. https://doi.org/10.1016/J.ANIREPROSCI.2016.09.011

Şen E, Tunali Y, Erkan M (2015) Testicular development of male mice offsprings exposed to acrylamide and alcohol during the gestation and lactation period. Hum Exp Toxicol 34:401–414. https://doi.org/10.1177/0960327114542883

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E et al (2020) Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front Physiol 11:694. https://doi.org/10.3389/FPHYS.2020.00694/BIBTEX

Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34:497–500. https://doi.org/10.1093/CLINCHEM/34.3.497

Takahashi M, Shibutani M, Inoue K, Fujimoto H, Hirose M, Nishikawa A (2008) Pathological assessment of the nervous and male reproductive systems of rat offspring exposed maternally to acrylamide during the gestation and lactation periods - a preliminary study. J Toxicol Sci 33:11–24. https://doi.org/10.2131/JTS.33.11

Takahashi M, Shibutani M, Nakahigashi J, Sakaguchi N, Inoue K, Morikawa T et al (2009) Limited lactational transfer of acrylamide to rat offspring on maternal oral administration during the gestation and lactation periods. Arch Toxicol 83:785–793. https://doi.org/10.1007/S00204-009-0418-Y

Tanaka H, Kasama T, Inomata K, Nasu F (1990) Abnormal movements in brindled mutant mouse heterozygotes: As related to the development of their offspring — Biochemical and morphological studies. Brain Dev 12:284–292. https://doi.org/10.1016/S0387-7604(12)80308-1

Traber MG (2008) Vitamin E and K interactions – a 50-year-old problem. Nutr Rev 66:624–629. https://doi.org/10.1111/J.1753-4887.2008.00123.X

Tse JKY (2017) Gut Microbiota, Nitric Oxide, and Microglia as Prerequisites for Neurodegenerative Disorders. ACS Chem Neurosci 8:1438–1447. https://doi.org/10.1021/ACSCHEMNEURO.7B00176

Tsukamoto M, Tampo Y, Sawada M, Yonaha M (2002) Paraquat-induced oxidative stress and dysfunction of the glutathione redox cycle in pulmonary microvascular endothelial cells. Toxicol Appl Pharmacol 178:82–92. https://doi.org/10.1006/TAAP.2001.9325

Wang H, Huang P, Lie T, Li J, Hutz RJ, Li K et al (2010) Reproductive toxicity of acrylamide-treated male rats. Reprod Toxicol 29:225–230. https://doi.org/10.1016/J.REPROTOX.2009.11.002

Wink DA, Hines HB, Cheng RYS, Switzer CH, Flores-Santana W, Vitek MP et al (2011) Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol 89:873–891. https://doi.org/10.1189/JLB.1010550

World Health Organization (2003) Acrylamide in drinking-water: background document for development of WHO guidelines for drinking-water quality. World Health Organization. https://apps.who.int/iris/handle/10665/75373

Yassa HA, George SM, Refaiy AERM, Moneim EMA (2014) Camellia sinensis (green tea) extract attenuate acrylamide induced testicular damage in albino rats. Environ Toxicol 29:1155–1161. https://doi.org/10.1002/TOX.21846

Yonar ME (2013) Protective effect of lycopene on oxidative stress and antioxidant status in Cyprinus carpio during cypermethrin exposure. Environ Toxicol 28:609–616. https://doi.org/10.1002/TOX.20757

Yousef MI, Abdallah GA, Kamel KI (2003) Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim Reprod Sci 76:99–111. https://doi.org/10.1016/S0378-4320(02)00226-9

Zaidi SMKR, Banu N (2004) Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. Clin Chim Acta 340:229–233. https://doi.org/10.1016/J.CCCN.2003.11.003

Zamani E, Shaki F, AbedianKenari S, Shokrzadeh M (2017) Acrylamide induces immunotoxicity through reactive oxygen species production and caspase-dependent apoptosis in mice splenocytes via the mitochondria-dependent signaling pathways. Biomed Pharmacother 94:523–530. https://doi.org/10.1016/J.BIOPHA.2017.07.033

Zhang Y, Zhang Y (2007) Formation and reduction of acrylamide in Maillard reaction: a review based on the current state of knowledge. Crit Rev Food Sci Nutr 47:521–542. https://doi.org/10.1080/10408390600920070

Zhang J, Yue W, Ren Y, Zhang C (2010) Enhanced role of elaidic acid on acrylamide-induced oxidative stress in epididymis and epididymal sperm that contributed to the impairment of spermatogenesis in mice. Toxicol Ind Health 26:469–477. https://doi.org/10.1177/0748233710373084

Zhao M, Zhang B, Deng L (2022) The Mechanism of Acrylamide-Induced Neurotoxicity: Current Status and Future Perspectives. Front Nutr 488. https://doi.org/10.3389/FNUT.2022.859189

Zingg JM (2018) Water-Soluble Vitamin E-Tocopheryl Phosphate. Adv Food Nutr Res 83:311–363. https://doi.org/10.1016/BS.AFNR.2017.12.007

Zirilli L, Rochira V, Diazzi C, Caffagni G, Carani C (2008) Human models of aromatase deficiency. J Steroid Biochem Mol Biol 109:212–218. https://doi.org/10.1016/J.JSBMB.2008.03.026