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Article

Lycopene modulates testicular injury oxidative stress and caspases upregulation induced by fenvalerate in male rats

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ABSTRACT

Fenvalerate (FEN) is one of the widely utilized synthetic pyrethroids that may negatively impact male fertility in animals and people, while the potential mechanism is still unknown. This study aimed to assess lycopene's potential therapeutic value in protecting male rats from testicular damage and oxidative stress caused by FEN. In our investigation, 28 male rats were randomized into four groups at random: lycopene (10 mg/kg BW), FEN (20 mg/kg BW), and lycopene plus FEN. The rats got their doses orally by gavage each day for four weeks. Animals that had consumed FEN showed high levels of hydrogen peroxide and thiobarbituric acid reactive substances, phosphatases, and aminotransferases. However, there was a considerable drop in antioxidant enzyme activity, glutathione, and protein content. Significant changes in testosterone, luteinizing and follicle-stimulating hormones, and sperm quality were also found. In addition, the expression of caspases 3 and 8 in rat testes was significantly upregulated. Biochemical, molecular, spermatological, and histological alterations have all been brought back to normal by lycopene. These findings imply that lycopene may have antioxidant and preventive properties against testicular damage brought on by FEN.

Keywords: Fenvalerate; Lycopene; Apoptosis; Semen quality; Oxidative stress.

INTRODUCTION

For many years, synthetic pyrethroids have been used as insecticides or repellents in homes and agriculture. Globally, they account for around a quarter of the market rate for pesticides[1]. Initially, it was thought that the most popular type II synthetic pyrethroid, fenvalerate, is not hazardous to mammals [2]. However, several studies suggest that the male reproductive system is damaged explicitly due to the buildup of fenvalerate in humans and animals[3]. A reduction in sperm characteristics and an induction in the fragmentation of DNA and chromosome aberration in spermatozoa are just a few of the fenvalerate adverse effects on semen quality [4]. The mechanism by which fenvalerate-induced poor semen quality is thought to be unknown. Possible reasons

involve reproductive endocrine disturbances, oxidative toxicity, and cell death[5]. Furthermore, it is generally accepted that normal and mature spermatogenesis depends on apoptosis regulation[6].

Lycopene, a carotenoid with significant antioxidant potential, is a red pigment in tomatoes and other fruits and vegetables [7]. Lycopene is one of over 600 different kinds of naturally occurring carotenoids and is an aliphatic hydrocarbon [8]. Due to its lengthy, acyclic shape, severe hydrophobicity, and conjugated double bonds, lycopene can conduct its antioxidant functions. Lycopene exhibits antioxidant properties by dissolving oxygen radicals and effectively removing them, like retinol, tocopherol, and carotenoids [9]. Additionally, among models of biological membranes, superoxide radicals that resemble liposomes are ideally eliminated by lycopene. Lycopene has potent antioxidant properties outside the cell, but it also works inside the cell to prevent the oxidation of DNA, proteins, and lipids. Lycopene may interact more effectively with the active oxygen species because it has eleven double bonds conjugated in its structure instead of β-carotene's nine [10]. So, the present work aims to investigate the potential protective influence of lycopene against FEN-caused testicular damage in rats.

MATERIALS AND METHODS

Chemicals

Fenvalerate (purity > 97%) was bought from Sigma Chemical Co (St. Louis, MO), and lycopene (10% FS Redivivo™) was supplied by DSM Nutritional Products (Heerlen, Netherlands).

Animals and treatment

Male Wistar rats (28 rats) weighing 150-170 g were acquired from Alexandria University's Faculty of Medicine, Alexandria, Egypt. The local committee at the university gave its approval to the experimental protocol in compliance with the National Institutes of Health's ethical standards. Seven animals were kept in each cage, fed a commercial meal, and given access to unlimited tap water. The animals were acclimated (temperature, 21°C; 40–60% relative humidity and 7 AM to 7 PM photoperiod) for two weeks. Four groups of animals were used: the first group was used as the reference group and given corn oil; the second group was supplemented with lycopene (10 mg/kg b.w.); the third group was treated with fenvalerate (20 mg/kg b.w.); and the fourth group administered with lycopene one hour before FEN treatment, respectively. Lycopene and fenvalerate were dissolved in corn oil and given daily and orally via gavages for 30 days, according to Velmurugan et al. (2004) [8] and El-Demerdash et al. (2004)[5], respectively. The animals were weighed and given isoflurane anesthesia, and blood and testicles were collected after the experimental time. The testes were cut into three slices, with the first slice being preserved in formalin (10%) for histology, the second was kept at -20°C for biochemical research, and the third was maintained at -80°C for molecular investigation.

Blood samples

Blood samples from a heart puncture were collected, allowed to stand for half an hour at 25°C, centrifuged at 3000g for 15 minutes, and then carefully separated the clear serum and kept at -20°C until it was required for additional experiments.

Hormones analysis

The blood testosterone (T) concentration was determined using a radioimmunoassay kit (RIA TESTO CTC KIT) provided by (Dia-Sorin Company: Stillwater, Minnesota, USA) while serum luteinizing hormone (LH) level was tested utilizing RIA kits bought from NIADDK, Bethesda, MD, USA, Follicle-stimulating hormone (FSH) levels were measured by immunodiagnostic techniques and the Elisa Kit test (DiaMetra kits, Via Giustozzi, Italy).

Sperms morphometric analysis

Each testicle's left caudal epididymis was gently removed, minced in 5 mL of Hanks's Buffered salt solution, and left at room temperature for 15 minutes to let spermatozoa flow into the fluid. The sperm parameters "sperm count, normality, motility, and viability" were evaluated utilizing a microscope (Olympus, Tokyo, Japan) and computer-assisted semen analysis, according to Adamkovicova et al. (2016) [11].

Tissue preparation

After the treatment phase, rats from each group were dissected. Each animal's testes were removed and homogenized in 10% w/v buffer containing 1.15% KCl and 0.01 mol/L sodium-potassium phosphate (pH 7.4). The supernatants were collected and used to evaluate the activity of several enzymes after the homogenates were centrifuged at 10,000 g for 20 min at 4°C.

Assessment of TBARS, H_2O_2, and GSH

By using the methods outlined by Ohkawa et al. (1979) and Velikova et al. (2000) [12], respectively, experiments on thiobarbituric acid-reactive compounds (TBARS) and hydrogen peroxide (H2O2) were carried out. At the same time, the Ellman method was used to measure the reduced glutathione (GSH) content[13].

Assessment of antioxidant enzyme activities

Superoxide dismutase activity (SOD; EC 1.15.1.1) was measured using Misra and Fridovich's method [14]. The catalase (CAT; EC 1.11.1.6) activity was determined using a double-beam spectrophotometer at 240 nm by estimating the rate of H_2O_2 degradation [15]. the activity of glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC 1.6.4.2) activities were assessed by the method of Hafeman et al. [16]. The activity of glutathione S-transferase (GST; EC 2.5.1.18) was assessed using para-nitrobenzyl chloride as a substrate[17].

Assessment of aminotransferases and phosphatases activity and protein content

The activities of aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), acid phosphatase (ACP; EC 3.1.3.2), and alkaline phosphatase (ALP; EC 3.1.3.1), as well as the protein content, were assessed using the commercially available kits supplied by Biodiagnostic Company, Egypt.

Molecular analysis using real-time PCR

Real-time PCR (qRT-PCR) was utilized to identify the relative expression of apoptotic genes (Caspase-3 and Caspase 8) in rat testis. Total RNA was extracted using a commercial kit (Gene JET RNA Purification Kit) according to the manufacturer's instructions (Thermo Scientific, # K0731, USA). The cDNA was built up by reverse transcription utilizing a commercial kit (RevertAid H Minus Reverse Transcriptase) following the manufacturer's instructions (Thermo Scientific, # EP0451, USA). The qPCR mixture contained cDNA, Syber green master mix (2x Maxima, Thermo Scientific, # K0221, USA), and primers. The reference gene was the

β-actin gene (internal control). Based on rat sequences obtained from GenBank databases, the primers were created using the web-based Primer 3 database (Table 1). The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ approach and displayed as a fold change.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Caspase3	GAGACAGACAGTGGAACTGACGATG	GGCGCAAAGTGACTGGATGA
Caspase8	AATGTTGGAGGAAAGCAATC	CATAGTCGTTGATTATCTTCAGC
β-actin	GGAGATTACTGCCCTGGCTCCTA	GACTCATCGTACTCCTGCTTGCTG

Table 1. The sequences of primers for qRT–PCR.

Histopathological investigation

According to the Bancroft and Stevens [18] method, testes were fixed in formalin's solution then successive paraffin sections were prepared to analyze the alterations in tissue architecture. Slides were examined under a light microscope and photographed (Olympus BX 41, Japan).

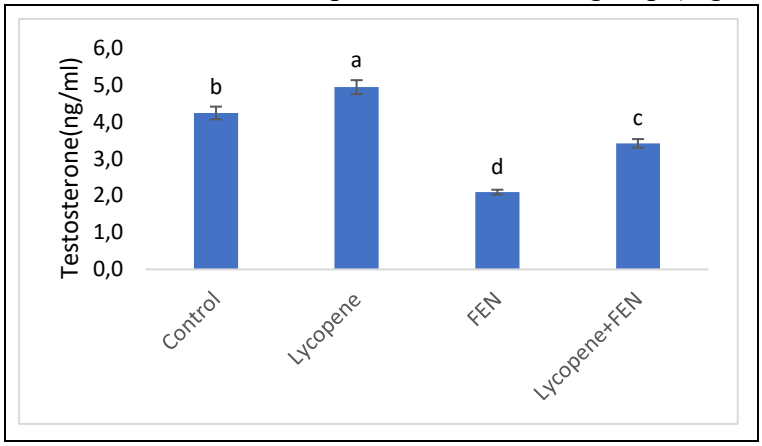
Statistical analysis

Data were all displayed as the mean ± standard error of the mean (SEM) and then analyzed by SPSS software (version 22, IBM Co., Armonk, NY). The means of various groups were compared using the one-way ANOVA and Tukey's post hoc testing. $P < 0.05$ was chosen as the significance level for each experiment.

RESULTS

Hormones level

According to the results, the testosterone, LH, and FSH levels in the rat serum of the fenvalerate-treated group were significantly different from those of the reference group. In comparison to the fenvalerate group, rats given lycopene and then treated with FNP had significantly higher hormone levels. In rats given lycopene alone, the hormonal levels were unaffected as compared to the control group (Figure 1).



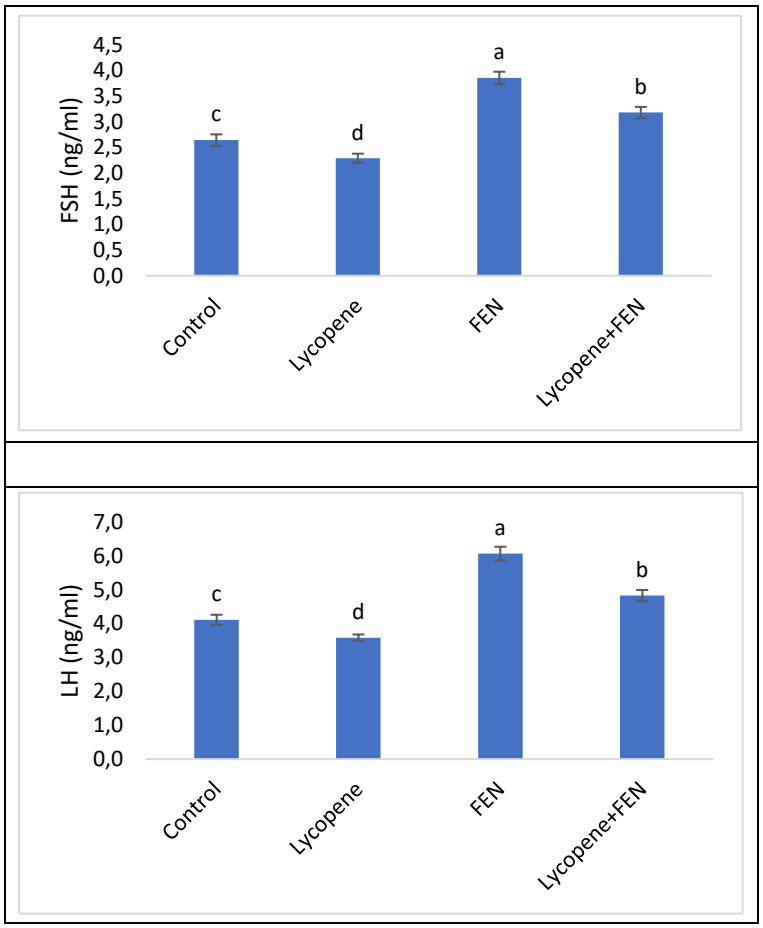


Figure 1. Hormone levels in different groups. Values are expressed as mean ± SE of seven rats per group. Columns with different letters are significantly other at p < 0.05. Groups are compared as follows: Lycopene and fenvalerate (FEN) groups are compared vs the control group, while the lycopene + fenvalerate group is compared vs the fenvalerate group.

Sperm parameters

When compared to control rats, animals given fenvalerate showed significantly ($P < 0.05$) reduced levels of sperm count, normalcy, motility, and viability. Rats pretreated with lycopene and then administered FNP showed better sperm quality than those given fenvalerate (Table 2).

Parameters	**Groups**			
	Cont.	Lycopene	Fenvalerate	Lycopene+ Fenvalerate
Sperm count (10^6 Cells)	85.80 ± 1.18^{b}	95.39 ± 1.05^{a}	24.74 ± 0.658^{d}	53.43 ± 1.57^{c}
Normal sperm %	81.99 ± 1.91^{a}	85.31 ± 1.91^{a}	28.00 ± 1.44^{c}	55.34 ± 1.61^{b}
Motility %	83.91 ± 3.10^{a}	90.20 ± 2.84^{a}	22.00 ± 0.54^{c}	51.07 ± 1.07^{b}
Viability %	56.43 ± 1.78^{b}	71.86 ± 0.800^{a}	25.43 ± 0.751^{d}	44.43 ± 0.948^{c}

Table 2. Sperm quality in rats of different groups.

Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, $p< 0.05$. Statistically significant variations are compared as follows: Lycopene and fenvalerate are compared to control while Lycopene + fenvalerate are compared to the fenvalerate group.

Oxidant stress and antioxidant biomarkers

The levels of TBARS and H_2O_2 were noticeably elevated. In contrast, SOD, CAT, GPx, GR, and GST activities, as well as GSH content, were dramatically lowered ($P< 0.05$) in the testes homogenate of rats exposed to fenvalerate as compared to controls. Additionally, animals given lycopene first and then FEN exhibited a considerable improvement in the previously evaluated indices compared to rats given FEN alone. Furthermore, compared to the control group, rats given lycopene solo showed a significant improvement of these indicators (Tables 3 and 4).

Experimental groups	Parameters		
	TBARS (nmol/g tissue)	H2O2 (µmol/g tissue)	GSH (mmol/mg protein)
Control	17.01±0.640c	48.65±0.707c	2.01±0.049b
Lycopene	13.47±0.501d	40.12±1.06d	2.36±0.061a
Fenvalerate	24.80±0.522a	68.69±2.357a	1.04±0.039d
lycopene + Fenvalerate	20.46±0.549b	60.38±1.746b	1.50±0.038c

Table 3. Effect of lycopene and fenvalerate and their combination on TBARS, H2O2, and GSH content levels in rats' testes.

Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Statistically significant variations are compared as follows: Lycopene and fenvalerate groups are compared vs the control group, while the lycopene + fenvalerate group is compared vs the fenvalerate group.

Experimental groups	Parameters				
	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GR (U/mg protein)	GST (µmol/hr/mg protein)
Control	$78.81±2.81^{b}$	$8.01±0.223^{b}$	$8.51±0.193^{c}$	$22.60±0.715^{b}$	$0.645±0.023^{b}$
Lycopene	$93.30±3.67^{a}$	$9.44±0.264^{a}$	$10.03±0.323^{a}$	$27.36±0.890^{a}$	$0.761±0.030^{a}$
Fenvalerate	$42.69±2.01^{d}$	$4.59±0.085^{d}$	$5.09±0.113^{d}$	$12.86±0.344^{d}$	$0.364±0.015^{d}$
Lycopene + Fenvalerate	$60.39±1.30^{c}$	$6.26±0.244^{c}$	$9.94±0.298^{b}$	$19.02±0.639^{c}$	$0.510±0.017^{c}$

Table 4. Effect of lycopene and fenvalerate and their combination on the activities of antioxidant enzymes in rat testes.

Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Statistically significant variations are

compared as follows: Lycopene and fenvalerate groups are compared vs the control group, while the lycopene + fenvalerate group is compared vs the fenvalerate group.

Aminotransferases, phosphatases activities, and protein content

The current research demonstrated that the protein content of rat testes decreased when compared to the control. At the same time, serum AST, ALT, ALP, and ACP activities were dramatically enhanced in rats treated with fenvalerate. In addition, animals given lycopene followed by FEN therapy as compared to the FEN group showed modulation in enzyme activities and protein concentration. Supplementing with lycopene did not have any discernible effects on its own (Table 5).

Experimental groups	Parameters				
	AST (U/l)	ALT (U/l)	ALP (U/l)	ACP (U/l)	Protein content (mg/g tissue)
Control	50.35±1.38c	56.89±1.45c	60.85±2.21c	9.59±0.430c	70.22±1.49a
Lycopene	52.95±1.51c	53.00±1.78c	57.00±2.51c	9.93±0.390c	73.00±1.74a
Fenvalerate	68.45±2.76a	77.69±2.630a	84.84±3.19a	13.44±0.466a	42.92±1.49c
Lycopene + Fenvalerate	56.00±0.612b	64.37±1.77b	69.43±2.97b	10.89±0.422b	57.92±1.58b

Table 5. Effect of lycopene and fenvalerate and their combination on serum AST, ALT, ALP and LDH activity and testes protein content in rats.

Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a column not sharing common superscript letters were significantly different, $p< 0.05$. Statistically significant variations are compared as follows: Lycopene and fenvalerate groups are compared vs the control group, while the lycopene + fenvalerate group is compared vs the fenvalerate group.

Expression of the caspase-8 and caspase-3

The expression of caspase-8 and caspase-3 was significantly increased in rats treated with fenvalerate compared to the control group. About the FEN group, rats administered lycopene and then given fenvalerate dramatically reduced the upregulation brought on by FEN. Additionally, rats given lycopene alone showed no significant difference compared to the control group (Table 6).

Experimental groups	Parameters	
	Caspase-3	Caspase-8
Control	1.00±0.04c	1.00±0.06c
Lycopene	0.93 ±0.14c	0.97±0.02c
Fenvalerate	7.84±0.26a	6.82±0.24a
lycopene + Fenvalerate	3.14±0.11b	1.53±0.05b

Table 6. Relative gene expression of caspase-3 and caspase-8 in different groups,

Histological observation of testis

Histopathological investigation of testicular sections from control (G1) and lycopene (G2) groups demonstrated plain testes structure, complete spermatogenesis, and a significant concentration of sperm in the lumen of the seminiferous tubules. In contrast, the testicles of the FEN group had diminished spermatogenesis-differentiated and wild lumen and visibly injured seminiferous tubules (G3). Seminiferous tubules have a damaged epithelial lining, many hyperchromatic spermatogenesis, a loss of differentiated spermatogenesis, necrotic spermatozoa with large lumens, and noticeably dilated interstitial tissue with atrophied Leydig cells. Rats who received both lycopene and FEN (G4) displayed well-differentiated stages of spermatogonia cells as spermatocytes and spermatid, as well as recovering seminiferous tubules surrounded by a thin basement membrane with Sertoli cells and prominent nuclei spermatogenic cells. Blood veins, Leydig cells, and many lymphocytes could be observed in the extensive interstitial tissue, while spermatozoa were apparent in the tiny lumina (Figure 2 and Table 7).

Parameters	Gp1	Gp2	Gp3	Gp4
Disorganized seminiferous tubules	+	++	++++	++
Characterize spermatogenic cells - Hyperchromatic - Necrotic	+++ + +	+++ ++ +	+ +++ +++	++ ++ ++
- Necrotic spermatocytes	+	+	+++	++
Dilated lumen	-	-	+++	++
Dilated interstitial tissue	+	+	++++	+++
Infiltrating lymphocytes	-	-	+++	++

Mild +, moderate ++, marked +++, sever ++++

Table 7. Histopathological changes in the testes tissue of rats in different experimental groups.

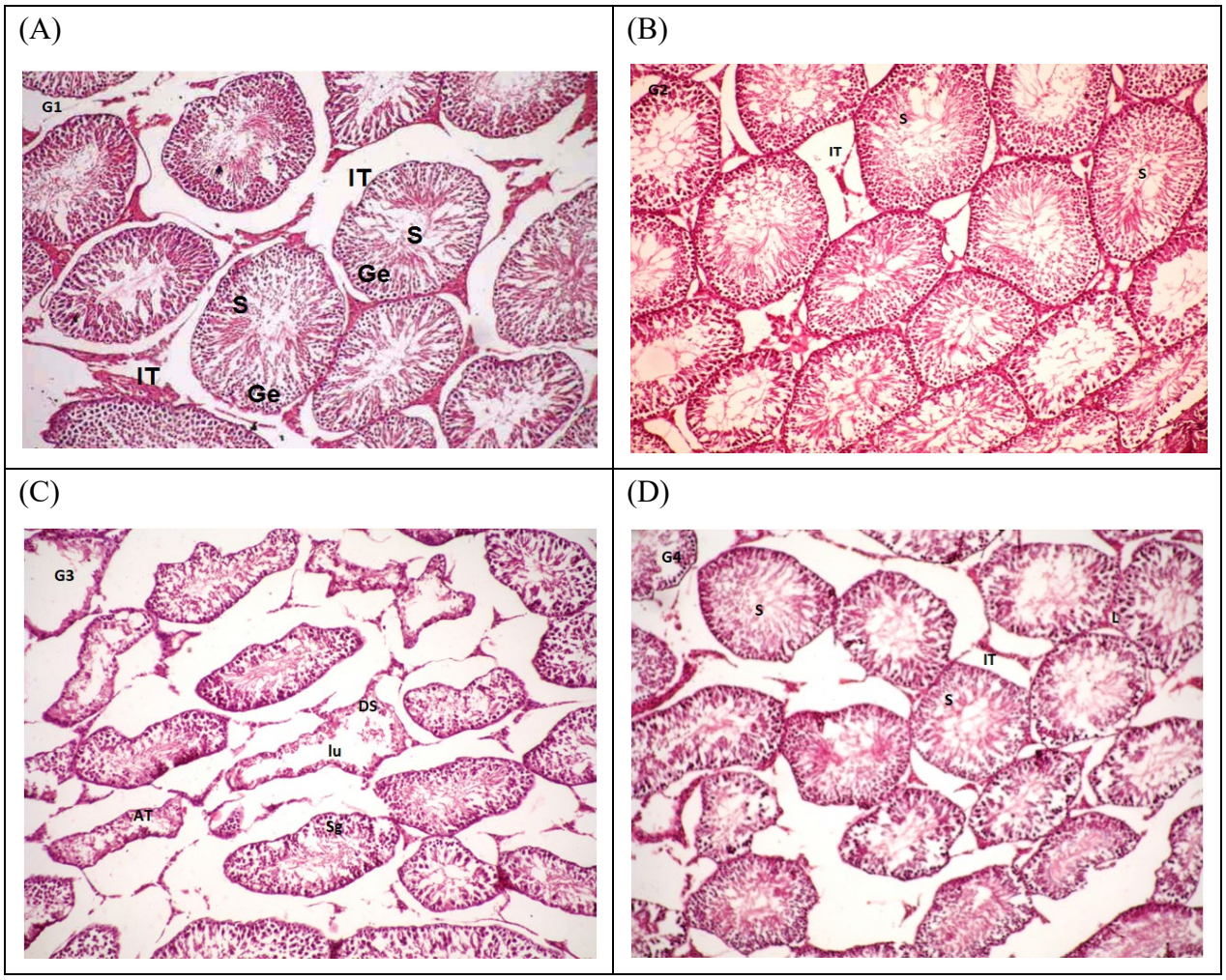


Figure 2. A&B: Rat testes sections in control (G1) and Lycopene (G2) groups revealed the standard structure of seminiferous tubules with a regular cycle of spermatogenesis. C: Testes sections of fenvalerate (G3) showed marked destructive seminiferous tubules with reduction of spermatogenesis differentiated (SP) and wide lumen. Marked dilated interstitial tissue and some atrophy of seminiferous tubules (AT). D: Testes sections in lycopene + fenvalerate group (G4) showed recovering seminiferous tubules (S) with redistributed lying spermatogenesis cells in different shapes and sizes and marked dilated interstitial tissue (IT) regulated its content. (H&E stain, 100XMagn).

DISCUSSION

It is crucial to find viable therapies for fenvalerate-induced reproductive damage since FEN exposure is a widespread public health issue. Several investigations indicated that lycopene was protective in the testes of rats exposed to contaminants such as polychlorinated biphenyl [19], bisphenol A [20], and Benzo [a]pyrene [21]. The observed drop in testosterone levels can be viewed as a bad thing for the quality of sperm. Also, our results show that the testicular tissue uses the increase in FSH and LH levels brought on by FEN treatment as a compensatory mechanism to prevent spermatozoa alterations and cellular damage [3]. According to the findings of Zhang et al. [22], exposure to fenvalerate significantly decreased the quality of semen, which might be connected to apoptosis of germ cells, DNA damage, a drop in testosterone levels, and cellular growth suppression. Fenvalerate also caused spermatogonial cell death and sterility in rats with damaged seminiferous tubules and testicular cells [10]. Furthermore, the large polyunsaturated fatty acids in spermatozoa's membranes make them susceptible to oxidative damage [23].

Because pyrethroids affect the hypothalamic-pituitary-testicular axis, they can change the levels of reproductive hormones [24]. Testicular toxicity appears to be significantly influenced by the testes' capacity to metabolize xenobiotic [25]. On the other hand, Cytochrome P450 performs a terminal oxidant role in several hydroxylation processes in testicular androgen production. A disruption in androgen metabolism and steroidogenesis caused changes in hormone levels after FEN treatment [26]. Testosterone is crucial for the beginning and maintenance of spermatogenesis. Hence, its decline was accompanied by a fall in spermatogenesis. Protein kinase A is activated by the binding of LH to the Leydig cells, which stimulates the cellular messenger cAMP, which in turn activates protein kinase A that undergoes a sequence of phosphorylations to activate enzymes required for testosterone production [27]. In addition, Sertoli cells phagocytose mature sperm in the absence of testosterone [28].

The antioxidant defense system protects cells against oxidative damage induced by xenobiotics due to its critical role in preserving the equilibrium of cellular function. According to numerous studies supporting our findings, pyrethroid insecticides like lambda-cyhalothrin and β-cyfluthrin produce free radicals and suppress the activity of antioxidant enzymes in vivo and in vitro [29]. A clear indication that FEN had the potential to produce oxidative damage was the observed rise in TBARS and H2O2 levels as well as the failure of antioxidant enzyme activities. An early effect of oxidative stress, manifested by rising TBARS and H2O2 levels, is a discernible decrease in GSH levels in testes homogenate. Type II pyrethroids are famed as secondary alcohol esters that have a cyano group at the a-carbon of the alcohol moiety [30]. Therefore, the liberation of the unstable cyanohydrins may cause FEN poisoning. The breakdown of cyanohydrins into cyanides and aldehydes could serve as free radicals source [29]. The current finding is consistent with earlier research that suggested the oxidative damage brought on by pyrethroids might be due to their lipophilicity, which allowed them to enter cell membranes 31 quickly.

Redox cycles of GSH play a crucial role in cellular antioxidant defenses and are essential for tissues to protect themselves against ROS damage. They take part in removing ROS by serving as a substrate for different enzymes like GPx and a nonenzymatic oxygen radical scavenger [32]. GSH might be created from GSSG by a reaction catalyzed by GR, or it could be formed independently, and oxidants, antioxidants, and growth factors [33] control this synthesis. GPx is a crucial enzyme for cellular antioxidant defense because it contains selenocysteine [34]. Under oxidizing circumstances, GSH is quickly converted to GSSG, which may then interact with the SH group of proteins to generate mixed disulfide via thiol/disulfide exchange. In the current study, oxidative stress caused by FEN exposure included suppression of the antioxidant defense system and increased LPO. Antioxidant enzymes play a crucial role in protecting living organisms from the harm caused by free radicals. Slight differences in the physiological levels of these antioxidant enzymes may significantly impact the resistance of cellular constituents to oxidative injury. Together with the nonenzymatic antioxidant GSH, these antioxidant enzymes work to disarm free radicals and stop oxidative cell damage [35]. The current findings showed that FEN significantly reduced the activities of CAT and SOD. There are two possible reasons for this drop. The first entailed the direct inhibition of SOD and CAT by FEN and the second implicated consumption throughout the breakdown of the high level of H2O2 and other free radicals or the inhibition of the enzyme activities by them. Additionally, the considerable reduction in the activity of GST in rat testes following FEN treatment may signify scanty detoxification and is likely connected to the reduction in GSH levels as it catalyzes the binding of a range of electrophilic substrates to the GSH thiol group, resulting in minimal toxic forms [36]. Accordingly, additional investigations have demonstrated that the overproduction of ROS caused by pyrethroid might upset the delicate balance between ROS production and antioxidant defenses[22]

In the FEN-treated group, the observed increase in AST, ALT, AcP, and ALP activity was amply

demonstrated, and they were discharged into the blood following cellular injury [37]. Lipid peroxidation weakens the permeability of cellular membranes, allowing cytoplasmic enzymes to leak out. [38]**.** According to Prasanthi et al. [39], fenvalerate can cause cellular damage and show several significant physiological disturbances, consistent with the current investigation. The biosynthesis of energy macromolecules, detoxification, and metabolic activities depend on phosphatases (ALP, ACP). The observed alteration in ALP and ACP activity agrees with the previous finding of Rahman et al. [40] due to phosphorothionate administration. Sugár et al. 41 state that the acid phosphatase enzyme is essential for various functions, such as "cellular metabolism, autolysis, differentiation, and dilatation of blood capillaries between seminiferous tubules ." Additionally, the increase in ALP activity in the testes may be brought on by cellular necrosis, which results in enzyme seepage into the bloodstream [40]. Lack of cellular function and alterations in the metabolism of protein is associated with a decrease in protein content [42] A histopathological study of the testes of FEN-exposed rats revealed abnormal architecture as a result of oxidative toxicity that caused apoptosis of germ cells, faint sperm quality, and modification in function and integrity of the gonads. In agreement, related research showed structural damage in pesticide-treated rat testes [43]. Additionally, according to Agarwal and Allamaneni (2006) [44], the degeneration of germ and Leydig's cells observed in the seminiferous tubule and interstitial tissues of the rats treated with fenvalerate was caused by ROS leading to apoptosis, which aids in the elimination of apparent germ cells from the testes preventing their output via caspases stimulation [45]. Moreover, prolonged exposure to pyrethroids, even at dosages that are significantly lower than the residual amounts found in the food consumed, will seriously impair both general physiological processes and reproductive function [46]

Lycopene is the most potent carotenoid antioxidant, shielding cells against ROS damage [47]. It is also a potent scavenger of 1O2 and other exciting species. Energy from 1O2 is transferred to the lycopene molecule during 1O2 quenching, changing it into the energy-dense triplet form. So, lycopene may offer protection against DNA, lipid, and protein oxidation in vivo[7]. Regarding defending the cell from the impacts of hydrogen peroxide and nitrogen dioxide radicals, lycopene has been proven to possess potent antioxidant activity among carotenoids.

Additionally, it has been discovered that lycopene has anticancer characteristics in vitro and in vivo and lessens the consequences of oxidative stress[8]. The current findings demonstrated that therapy with lycopene alone resulted in significant modifications in some of the measured parameters and a significant increase in the antioxidant defense system. On the other hand, lycopene induced worthy amendments in lipid peroxidation marker levels in rats treated with FEN. Lycopene's potential as an antioxidant against xenobiotics may cause its positive effects [7]. Additionally, the improvement in sperm quality is consistent with previous reports that lycopene has been proposed as a substitute therapy for sperm damage following chemotherapy (18). Ma et al. (2018) [48] hypothesized that supplementation with lycopene reversed the harmful effects of BPA on the reproductive system in a study on young mice. Furthermore, according to Boeira et al. (2015) [49], lycopene likely mediates antioxidant and anti-inflammatory actions. As a result, it may have assisted in reversing the impairment of testicular functions caused by zearalenone and permitted testosterone levels to rise once more.

The observed reduction in the concentration of free radicals that lycopene prevents is connected to the reported induction of antioxidant enzymes. Additionally, reduced glutathione is a crucial nonenzymatic antioxidant essential for preserving cell integrity because of its thickening properties and role in cell metabolism. According to Bose and Agrawal (2007) [50], lycopene administration increased GSH levels, which are crucial for maintaining high GPx and GST activities. Lycopene also moderated the harmful effects of FEN due to its high antioxidant activity and free radical scavenging capacity[19]. Increased GSH content detoxifies reactive

chemicals through the glutathione redox cycle and guards cellular proteins against oxidation. It can be concluded that lycopene's antioxidant characteristics may be responsible for its ability to reduce the testicular toxicity caused by FEN successfully.

CONCLUSIONS

The current finding demonstrated that fenvalerate caused rat testicular injury. Apoptosis, hormonal abnormalities, oxidative stress, issues with enzymatic and nonenzymatic antioxidants, and poor sperm quality are all possible effects. Additionally, before fenvalerate treatment, lycopene supplementation modifies its harmful effects by enhancing antioxidant status, reducing ROS production, and improving testicular functioning due to its potent antioxidant activity. So, lycopene may be a potential agent with a protective effect that can be used to repair the testis damage caused by fenvalerate in rats.

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Author Contributions:

Jebur A.B.: Conceptualization, Practical Work, Data generation, Writing - Original Draft, Publication.

Raghda AE: Conceptualization, Practical Work, Data Analysis, Manuscript revision.

El-Demerdash F.M.: Conceptualization, Methodology, Manuscript revision, and investigation**.**

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Informed Consent Statement: Any research article describing a study involving humans should contain this statement "Not applicable." for studies not involving humans.

Data Availability Statement

The data used to support the findings of this study are included in the article.

Conflicts of Interest: The authors declare that they have no conflicts of interest regarding the publication of this paper**.**

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