The Effects of Vitamins C and E against Chlorpyrifos Toxicity on *Capra hircus* Spermatozoa: Involvement of Oxidative Stress

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Abstract. *Chlorpyrifos (CPF), a widely used broad-spectrum pesticide, has been shown to affect significantly fertility outputs. However, there is limited knowledge concerning the potential involvement of oxidative stress (OS) in CPF-induced sperm dysfunction in mature spermatozoa and the alternatives to counteract the potential deleterious effects. This study aimed, on the one hand, to investigate the effects of CPF on sperm motility and to assess whether OS may be involved in CPF-induced toxicity in epididymal spermatozoa. On the other hand, it aimed to evaluate the effects of vitamin C and vitamin E, administered individually or in association in the presence of CPF. Epididymal sperm was collected from five Capra hircus testes and divided into five equal-volume aliquots, receiving different treatments: Control–, CPF, VitC+CPF, VitE+CPF and VitCE+CPF. Control– group was diluted in TRIS extender, while CPF group was exposed to CPF (50 µg/mL). The vitamin-treated groups were pre-incubated for 20 min with vitamin C (0.1 mg/mL), vitamin E (0.25 mg/mL), or their combination prior to CPF exposure. Sperm motility was assessed after 0, 30, and 60 min of storage at 37°C using computer aided sperm analysis. Malondialdehyde (MDA) levels were measured at 30 min in each treatment by the thiobarbituric acid reactive substances (TBARS) assay and quantified at 535 nm. CPF exposure significantly impaired sperm kinetics; at 60 min, VSL decreased from 11.248 ± 0.209 µm/s in the Control– group to 5.051 ± 0.143 µm/s in the CPF group ($P < 0.0001$). Also, progressive motility decreased in the CPF group (0.225 ± 0.084%) compared with the Control– group (4.146 ± 1.224%). VitCE+CPF improved significantly VSL compared with CPF group (17.405 ± 0.185 µm/s vs CPF; $P < 0.0001$). MDA levels were elevated significantly in the CPF group (2.261 ± 0.108 nmol MDA/10^8 SPZ) compared with the Control– group (1.919 ± 0.109 nmol MDA/10^8 SPZ; $P = 0.035$). VitCE+CPF resulted in the lowest MDA concentration (1.739 ± 0.107 nmol MDA/10^8 SPZ vs CPF; $P = 0.003$). The results showed that CPF affected mature spermatozoa, accompanied by an increase in lipid peroxidation. These effects may be attenuated in the presence of vitamins C and E, especially when they are used in association.*

Introduction

Pesticides find extensive application in agriculture and industry owing to their capacity to protect crops during growth from insects, weeds, fungi, and bacteria (Gangola et al., 2022; Sule et al., 2022). However, they are considered one of the most important contaminants worldwide due to their persistence and toxicity, and numerous studies have reported their detrimental effects on the environment and human health (Gangola et al., 2022; González-Curbelo et al., 2022). Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothionate) (CPF), an organophosphate pesticide, is one of the most widely used broad-spectrum pesticides and is classified as a moderately hazardous class II insecticide (Alaa-Eldin et al., 2017). It is mainly used in agriculture to control both foliage, soil-borne pests affecting crops, flea treatments, and in residential settings (Sabarwal et al., 2018; Singh et al., 2018). Many epidemiological studies have reported a correlation between exposure to organophosphorus (OP) molecules and reproductive disorders (Dhanushka and Peiris, 2017; Chhillar et al., 2023). In vivo, Alaa-Eldin et al. (2017) found that chlorpyrifos and cypermethrin induced reproductive toxicity in albino rats affecting testicular weight, sperm motility, viability, and count. Similarly, Joshi et al. (2007) reported that subchronic exposure to CPF at doses of 7.5, 12.5, and 17.5 mg/kg/day for 30 days altered male rats' fertility by causing severe testicular damage, declining sperm count and motility and decreasing serum testosterone levels. These findings were further supported by Farag et al. (2010), who reported similar results in male mice treated with CPF at 15 or 25 mg/kg/day for 4 weeks.

It has been established that OP pesticides exert their toxicity mainly by inhibiting acetylcholinesterase,

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resulting in the accumulation of acetylcholine at the cholinergic nerve terminal (Dhanushka and Peiris, 2017; Lushchak et al., 2018). Other putative mechanisms, including oxidative stress (OS), have also been reported. In particular, chlorpyrifos may induce OS by generating free radicals, altering antioxidant defense (Uchendu et al., 2018), and consequently inducing reproductive toxicity (Mandal and Das, 2011). In fact, it has been highlighted that OS is considered a potential cause of male infertility (Latchoumycandane et al., 2002; Altaher et al., 2023), causing substantial damage during spermatogenesis and leading to lipid, protein, and DNA alterations (Moridi et al., 2018; Altaher et al., 2023). Recently, Talebinasab et al. (2025) have found that male rats exposed to CPF exhibited testicular toxicity with increased MDA levels and decreased activity of antioxidant enzymes.

Numerous researchers have investigated various approaches to overcome such adverse effects on reproductive function. In particular, antioxidant supplementation has been highlighted as a promising approach to mitigate the damaging effects of pesticides. Antioxidants serve as agents that eliminate, scavenge, and inhibit the generation of ROS (Sheweita et al., 2005). However, as the endogenous antioxidant system may not be sufficient to protect cells from OS, antioxidant supplementation is widely considered an effective alternative (Sule et al., 2022). Thus, vitamin E, also known as α-tocopherol, and vitamin C have emerged as effective scavengers of ROS (Mukai et al., 1993; Bhardwaj et al., 2018). In this regard, Aly et al. (2010) suggested that pre- or post-administration of vitamin C in male mice exposed to CPF prevented oxidative damage. Jaiswal et al. (2013) also showed that vitamin C supplementation significantly reduced OS markers in the hearts of rats exposed to carbofuran. However, the association of vitamins C and E appears to provide better protection against OS damage in different experimental reports. In this respect, vitamins C and E have been shown to improve pregnancy rates in couples (Zhou et al., 2022) and to protect spermatogenesis against endosulfan (Takhshid et al., 2012).

However, to the best of our knowledge, no previous studies have investigated either the direct impact of chlorpyrifos (CPF) on mature spermatozoa using an in vitro model or the potential effects of vitamins C and E on CPF-induced sperm alterations. The present study aimed first to assess CPF's effects on sperm parameters without considering other associated factors, including hormonal secretion or testicular integrity, which may be involved in vivo. Second, the study aimed to investigate whether oxidative stress may contribute to CPF-induced toxicity, as assessed by lipid peroxidation, and to examine the effects of vitamins C and E, administered individually or in association.

Materials and Methods

Ethical Statement

Testes from adult *Capra hircus* were obtained from a local slaughterhouse following routine slaughter. The study involved only in vitro experiments on semen samples and did not require direct animal experimentation. All procedures were conducted in accordance with institutional guidelines and good veterinary practice.

Semen Collection and Preparation

Testes were collected from five adult goats (*Capra hircus*, two years old) obtained from a local slaughterhouse in Béjaïa (Algeria). One testis from each animal was used in the present study (N = 5). Immediately after slaughter, testes were collected and transported to the laboratory in a sterile airtight container maintained at approximately 37°C. The experimental procedures were initiated within 1 h post-slaughter. Semen was obtained by the retrograde flushing method as previously described by Martinez-Pastor et al. (2006). The epididymis was meticulously isolated from the testis and rinsed using a TRIS extender. An incision was made at the cauda of the epididymis, and measured pressure was applied through the introduction of 1 mL of TRIS extender into the lumen of the vas deferens.

Chemicals

All chemicals, including Tris(hydroxymethyl) aminomethane, fructose, citric acid, polyethylene glycol 6000, trichloroacetic acid (TCA), and thiobarbituric acid (TBA), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Vitamin C (ascorbic acid) and vitamin E (α-tocopherol) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). The chlorpyrifos used in this study was PYRICAL 480 EC, which is a concentrated emulsifiable formulation containing 480 g/L of chlorpyrifos, supplied by ARYSTA Life Science.

In Vitro Exposure of Spermatozoa to Chlorpyrifos/Pre-Incubation with Vitamins

In this study, a TRIS extender composed of 1.510 g Tris(hydroxymethyl)aminomethane, 0.625 g fructose, 0.850 g citric acid, 0.050 g penicillin G dissolved in 50 mL of distilled water was used for semen dilution.

Each semen sample was divided into five equal-volume aliquots (10 µL) to evaluate the effects of five experimental treatments:

- Control– served as the negative control and contained only TRIS extender.
- CPF served as the positive control, receiving CPF exposure without antioxidant supplementation.
- VitC+CPF was supplemented with vitamin C (0.1 mg/mL; 0.57 mM) 20 min before exposure to CPF.
- VitE+CPF was supplemented with vitamin E

(0.25 mg/mL; 0.58 mM) 20 min before exposure to CPF.

- VitCE+CPF was supplemented with a combination of both vitamins, vitamin C (0.1 mg/mL; 0.57 mM) and vitamin E (0.25 mg/mL; 0.58 mM), 20 min before exposure to CPF.

The experimental design consisted of two sequential dilution steps. In the first step (1:100), 10 µL of fresh semen was added to 990 µL of treatment medium (TRIS extender or vitamins), and the mixture was incubated at 37°C for 20 min. A second dilution step (1:2) was then performed by adding CPF (50 µg/mL) (Pallotta et al., 2018) to corresponding groups, except the Control–.

To enhance its solubility, vitamin E was solubilized in polyethylene glycol 6000 (PEG 6000) at a 10:90 (w/w) ratio, as described by Amokrane et al. (2020).

Computer-assisted Sperm Analysis (CASA)

Sperm analysis was performed in each sample at 0, 30, and 60 min using the Sperm Class Analyzer® (SCA) Version 5.4 (Microptic S.L. Viladomat 321, 6e408029, Barcelona, Spain). For each sample, 10 µL of sperm was analyzed using an analysis chamber with a depth of 10 µm (Makler Counting Chamber, Sefi-Medical Instruments Ltd., Biosigma S.r.l., Italy) maintained at 37°C. Acquisition was performed at 25 frames per second with a 10× objective using a phase contrast microscope. The standard CASA settings included a head area range of 3–70 µm^2. Spermatozoa were considered motile when curvilinear velocity (VCL) exceeded 10 µm/s. The sperm kinetic parameters evaluated were curvilinear velocity (VCL, µm/s), average path velocity (VAP, µm/s), and straight-line velocity (VSL, µm/s). Sperm motility parameters included progressive motility (PM, %) and rapid motility (R, %).

Measurement of Malondialdehyde

The malondialdehyde (MDA) level was assessed in each treatment as an indicator of lipid peroxidation using the thiobarbituric acid (TBA) assay as described by Buege and Aust (1978), adapted by introducing sperm washing, standardization of sperm concentration (100×10^6 spermatozoa/mL), as well as reagent volumes and centrifugation conditions. Absorbance was measured using a Jenway Genova spectrophotometer (Jenway Ltd., Essex, UK). Prior to TBARS analysis, sperm concentration in each sample was determined by CASA and adjusted to 100×10^6 spermatozoa/mL to ensure comparability among treatments.

Briefly, after 30 min of co-incubation, all samples were centrifuged at 800 × g for 10 min to separate the spermatozoa from the treatment media. Distilled water was added to the pellets, and the samples were centrifuged at 6000 × g for 15 min. Then, 315 µL of trichloroacetic acid (TCA) and 125 µL of TRIS extender were added to each supernatant. All samples were incubated at 0°C for 2 h, then centrifuged at 16 000 × g for 10 min. Subsequently, 150 µL of TBA was added to the supernatant of each sample.

The samples were boiled for 15 min and then cooled in an ice bath. Finally, absorbance was measured at 535 nm. The molar extinction coefficient for MDA was 1.56×10^5 M^{-1} cm^{-1}. The results were expressed as nmol MDA/10^8 SPZ.

Statistical Analysis

Data were collected and expressed as mean ± SEM. Normality of data distribution was assessed prior to statistical analysis for all parameters. Kinetic parameters (VCL, VAP, VSL) did not satisfy the normality assumption ($P < 0.05$) and were therefore analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U test with Bonferroni corrections performed in Stat View (SAS Institute Inc.). Progressive (PM), rapid (R) motility, and MDA levels satisfied the normality assumption ($P > 0.05$) and were therefore analyzed using ANOVA (GraphPad Prism, version 8.0.2). Two-way ANOVA followed by Dunnett's multiple comparisons test was applied to PM and R, assessed at three time points (0, 30, and 60 min), with the CPF group as reference. One-way ANOVA with Dunnett's multiple comparisons test was applied to MDA levels, measured at a single time point (30 min), with the CPF group as reference. Differences were considered statistically significant at $P < 0.05$.

Results

Kinetic Parameters of Spermatozoa Co-Incubated with Chlorpyrifos and Vitamins

CPF exposure significantly reduced VCL, VAP, and VSL compared with the Control– group ($P < 0.0001$), whereas treatment with vitamin C, vitamin E, or their association significantly increased these parameters compared with the CPF group ($P < 0.0001$) as shown in Fig. 1.

Among the vitamin-treated groups, the combined group VitCE+CPF showed the highest VCL, VAP, and VSL values across the 0, 30, and 60 min assessments.

At 60 min, the VitCE+CPF group showed VCL, VAP, and VSL values of 70.110 ± 0.522, 31.563 ± 0.231, and 17.405 ± 0.185 µm/s, respectively. In contrast, the CPF group showed corresponding values of 30.347 ± 0.507, 15.095 ± 0.247, and 5.051 ± 0.143 µm/s, respectively. Both VitC+CPF and VitE+CPF significantly improved sperm kinetic parameters compared with the CPF group ($P < 0.0001$). Mean values are summarized in Table 1.

Effects on the Percentages of Progressive and Rapid Motility

Fig. 2 illustrates the percentages of progressive (PM) and rapid (R) motility in the five experimental groups at 0, 30, and 60 min. CPF exposure significantly decreased rapid motility compared with the Control–

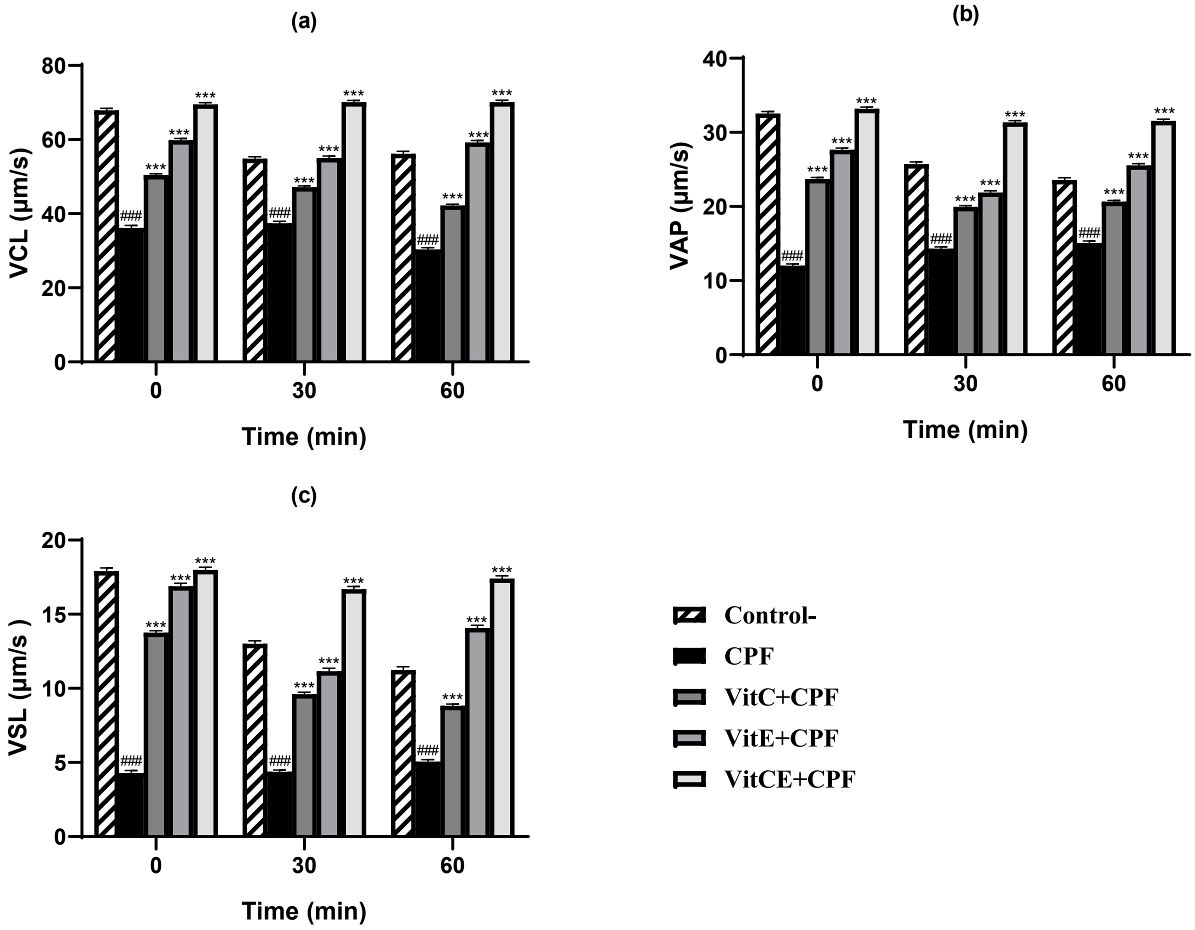

Fig. 1. Kinetic parameters of spermatozoa co-incubated with chlorpyrifos and vitamins. (mean ± SEM) values for (a) curvilinear velocity (VCL), (b) average path velocity (VAP), (c) straight-line velocity (VSL), at 0, 30, and 60 min in the five tested groups

$^{\#}$ Significant difference between CPF and Control–. * Significant difference between vitamin-treated groups and CPF. $^{\#}$ $P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 1. Comparative values (mean ± SEM) of sperm kinetic parameters at the 60 min evaluation time point

	Control–	CPF	VitC+CPF	VitE+CPF	VitCE+CPF
VCL (µm/s)	56.186 ± 0.676	30.347 ± 0.507$^{\#\#\#}$	42.232 ± 0.328***	59.236 ± 0.551***	70.110 ± 0.522***
VAP (µm/s)	23.571 ± 0.311	15.095 ± 0.247$^{\#\#\#}$	20.641 ± 0.161***	25.549 ± 0.245***	31.563 ± 0.231***
VSL (µm/s)	11.248 ± 0.209	5.051 ± 0.143$^{\#\#\#}$	8.831 ± 0.112***	14.071 ± 0.192***	17.405 ± 0.185***

$^{\#}$ Significant difference between CPF and Control–. * Significant difference between vitamin-treated groups and CPF. $^{\#}$ $P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

at all time points (0 min: $P = 0.0009$; 30 min: $P = 0.012$; 60 min: $P = 0.032$). The same trend was observed in progressive motility, with a significant reduction only at 0 min ($P = 0.015$). No significant differences were observed at 30 and 60 min.

Among the vitamin-treated groups, the VitCE+CPF group showed higher PM and R values compared with the CPF group. At 60 min, VitCE+CPF showed PM and R values of 9.112 ± 3.357% and 29.374 ± 9.379%, respectively, compared with 0.225 ± 0.084% and 1.365 ± 0.232% in the CPF group. However, these differences were not statistically significant. In contrast, the VitE+CPF group showed a significant improvement in PM and R compared with the CPF (PM: $P = 0.018$; R: $P = 0.004$). No significant differences were observed for the VitC+CPF group. Mean values are summarized in Table 2.

Lipid Peroxidation

Lipid peroxidation was assessed after 30 min of co-incubation in all groups. As shown in Fig. 3, MDA levels differed significantly among the experimental groups. Compared with the Control– group, MDA levels were significantly higher in the CPF group ($P = 0.035$). Comparative values (mean ± SEM) are presented in Table 3.

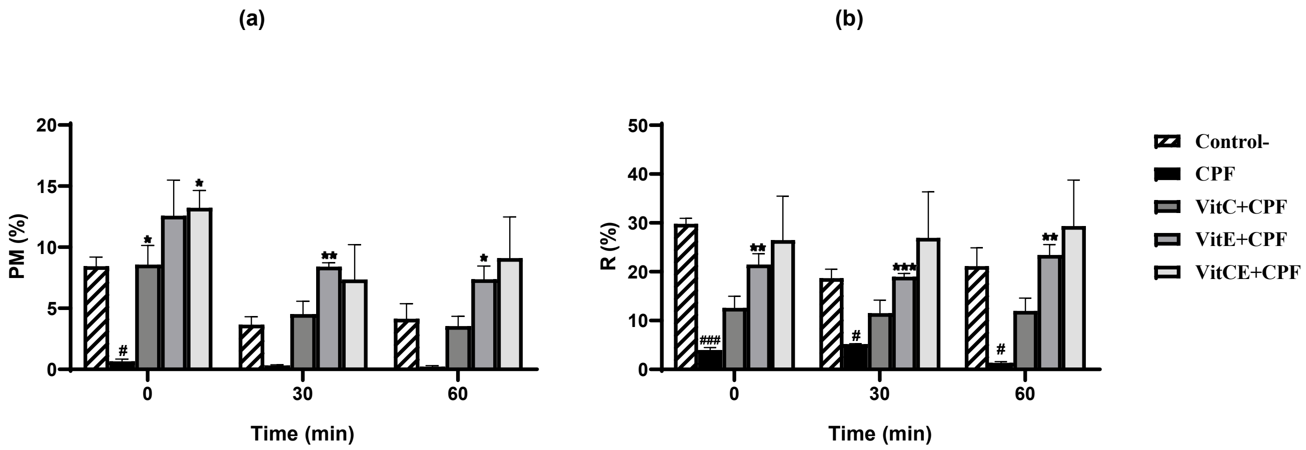

Fig. 2. Percentages (mean ± SEM) of progressive motility (PM) (a) and rapid motility (b) at 0, 30, and 60 min in the five groups

$^{\#}$ Significant difference between CPF and Control–. * Significant difference between vitamin-treated groups and CPF. $^{\#}$ $P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Absence of symbols indicates no statistically significant difference from CPF ($P > 0.05$).

Table 2. Comparative values (mean ± SEM) of progressive motility (PM) and rapid motility (R) at the 60 min evaluation time point

	Control–	CPF	VitC+CPF	VitE+CPF	VitCE+CPF
Progressive motility (%)	4.146 ± 1.224	0.225 ± 0.084	3.542 ± 0.807	7.371 ± 1.084*	9.112 ± 3.357
Rapid motility (%)	21.153 ± 3.749	1.365 ± 0.232$^{\#}$	11.967 ± 2.632	23.446 ± 2.134**	29.374 ± 9.379

$^{\#}$ Significant difference between CPF and Control–. * Significant difference between vitamin-treated groups and CPF. $^{\#}$ $P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Absence of symbols indicates no statistically significant difference from CPF ($P > 0.05$).

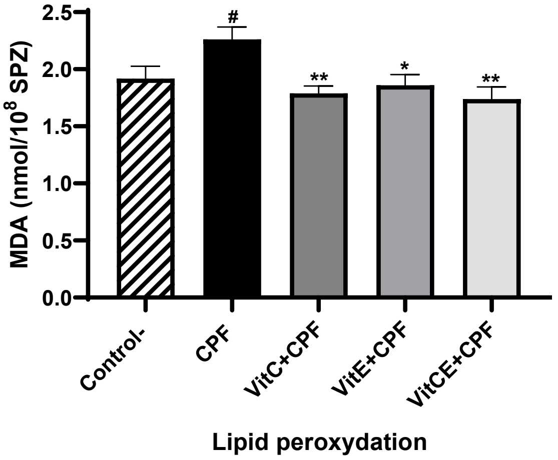

Fig. 3 Malondialdehyde (MDA) levels Values are presented as mean ± SEM.

$^{\#}$ Significant difference between CPF and Control–. * Significant difference between vitamin-treated groups and CPF. $^{\#}$ $P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Comparative values (mean ± SEM) of malondialdehyde (MDA) concentration at the 30 min evaluation time point

	Control–	CPF	VitC+CPF	VitE+CPF	VitCE+CPF
MDA levels (nmol MDA/10^{8} SPZ)	1.919 ± 0.109	2.261 ± 0.108$^{\#}$	1.788 ± 0.066**	1.860 ± 0.092*	1.739 ± 0.107**

$^{\#}$ Significant difference between CPF and Control–. * Significant difference between vitamin-treated groups and CPF. $^{\#}$ $P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

MDA levels were decreased in the vitamin-treated groups, with the lowest value in the VitCE+CPF group (1.739 ± 0.107 nmol MDA/10^8 SPZ); compared with the CPF group ($P = 0.003$). Significant differences were noted between VitC+CPF ($P = 0.007$) and VitE+CPF ($P = 0.029$) groups compared with the CPF group.

Discussion and Conclusions

Male reproductive function is susceptible to a broad spectrum of chemicals, particularly pesticides (Ashiru and Odusanya, 2009), which have received significant research attention in recent decades. Several toxicological studies have indicated that chlorpyrifos (CPF) can be harmful to various mammal species, affecting multiple organs and tissues. It has been especially reported that reproductive function was affected at different levels, impairing hormone secretion and testicular integrity (Moreira et al., 2021; Darwish et al., 2025). The use of in vivo models has revealed significant impairment in all sperm parameters. However, it remains unclear whether this impairment is related to hypothalamic-pituitary-gonadal axis dysregulation and testicular impairment or to a direct impact on mature spermatozoa. In this context, the present study aimed to assess the direct in vitro impact of CPF on mature spermatozoa, to evaluate whether oxidative stress may contribute to CPF-induced toxicity as assessed by lipid peroxidation, and to investigate the effects of vitamins C and E, either individually or in association.

Kinetic parameters, including VCL, VAP, and VSL, as well as progressive and rapid motility, were evaluated in all experimental groups. The results showed a notable decrease in all sperm motility parameters in the CPF-treated group, whereas the groups receiving vitamins C and E exhibited higher values than the CPF group, particularly when they were associated. In addition, CPF significantly increased MDA levels, indicating enhanced lipid peroxidation. This increase was reduced in the presence of vitamins C and E or their association. These findings are consistent with in vivo studies reporting that CPF induces complete loss of motility in male mice, along with a concomitant increase in OS (Zhang et al., 2020). Furthermore, CPF has been associated with lipid peroxidation in testicular membranes and oxidative stress production (Mandal and Das, 2011). In fact, OS has been proposed as a key mechanism underlying the toxicities of several pesticides, including organophosphates (Abdollahi et al., 2004). In a comparable in vitro study on caprine testicular cells, Bhardwaj et al. (2018) assessed oxidative stress induced by cypermethrin by measuring lipid peroxidation (TBARS) and ferric reducing antioxidant power (FRAP), alongside antioxidant enzyme activities including catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST).

Mammalian sperm cells are highly susceptible to oxidative damage owing to their high content of polyunsaturated fatty acids, which are prone to peroxidation (Sheweita et al., 2005; Wathes et al., 2007; Chianese and Pierantoni, 2021). In this context, docosahexaenoic acid (DHA), a major fatty acid in sperm cell membranes, is especially vulnerable to lipid peroxidation due to its six double bonds (Wright et al., 2014). This can result in elevated production of reactive oxygen species (ROS), leading to increased sperm DNA fragmentation and mitochondrial dysfunction, ultimately contributing to male infertility (Kumar and Singh, 2022). Moreover, spermatozoa contain a large number of mitochondria due to their constant energy requirements for optimal motility (Darmawan, 2007). These mitochondria, as an energy source, are significantly affected by the integrity of the sperm's tail and midpiece (Talwar and Hayatnagarkar, 2015; Chianese and Pierantoni, 2021). In fact, Zhang et al. (2020) have shown a decrease in sperm motility in mice induced by CPF associated with decreased mitochondrial activity.

Several antioxidants, including vitamin C, vitamin E, glutathione, and coenzyme Q10, are recognized for their efficacy in treating male infertility (Sheweita et al., 2005). Regarding the vitamin treatment, results showed that both vitamins C and E were associated with attenuation of the CPF-induced impairment of sperm motility, especially when associated. Previous studies have demonstrated that antioxidant supplementation significantly improves sperm parameters. Indeed, Zalata et al. (2014) reported the protective effects of vitamins C (20 mM) and E (2 mM) on human spermatozoa exposed to cypermethrin. Bhardwaj et al. (2018) have also demonstrated that vitamins C and E at 0.5 and 1.0 mM reduced cytotoxicity and oxidative stress in the testicular tissue of male caprine exposed to cypermethrin. These findings are consistent with our findings.

Vitamin E, a lipophilic antioxidant, acts as a chain-breaking agent in lipid peroxidation by scavenging peroxyl and alkoxyl radicals, thereby preventing free radical amplification (Hammadeh et al., 2009). Vitamin C (ascorbic acid), a hydrophilic antioxidant, neutralizes free superoxide, peroxyl, and hydroxyl radicals (Wefers and Sies, 1988). The difference between vitamin E and vitamin C may be attributed to vitamin E's lipophilic nature, which facilitates its free distribution within cell membranes (Takhshid et al., 2012). The combination of vitamins C and E was associated with more attenuation of CPF-induced toxicity, compared with vitamins alone. This may be related to the capacity of vitamin C to regenerate the oxidized form of vitamin E, thereby optimizing antioxidant defence (Wefers and Sies, 1988).

In conclusion, the current in vitro study demonstrates that chlorpyrifos significantly impairs sperm kinetic parameters and increases lipid peroxidation in mature epididymal spermatozoa. This suggests that oxidative stress may contribute to CPF-induced sperm toxicity. Pre-treatments with vitamins C and E attenuate these effects, particularly when they are associated.

However, several limitations in the present study should be acknowledged. First, oxidative stress was assessed using only a single marker of lipid peroxidation (MDA). Therefore, future studies should include additional oxidative stress biomarkers, such as SOD, CAT, and GSH activities, to provide a more comprehensive evaluation of oxidative stress involvement. Second, the relatively limited sample size (N = 5) may limit the generalizability of the findings to the broader caprine population. Furthermore, expressing results as mean ± SEM, while useful for comparing treatment groups, does not fully reflect inter-individual variability. Also, the absence of vitamin-only control groups limits the distinction of the intrinsic effects of vitamins C and E from CPF-related interactions. Future investigations should involve larger sample sizes, multiple oxidative stress markers, and appropriate vitamin-only control groups to strengthen the current results. In addition, artificial insemination studies could reveal the real interest of such vitamin protection on fertility outputs.

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