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Effects of Pyrethroid Exposure on Testicular Histopathology and Biochemistry in Adult Male Wistar Rats: Evaluation of Ascorbic Acid and Alpha-Tocopherol Administration

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ABSTRACT

Background: The application of pyrethroid pesticides to control pests has been widely implemented both domestically and industrially in Nigeria. Excessive and unregulated usage of pesticides can have toxic effects on the male reproductive system. **Objectives:** This study investigated the toxicological impact of Cypermethrin on the testes, as well as the potential mitigating effects of single and combinatorial administration of ascorbic acid and alpha-tocopherol in adult male Wistar rats. **Methods:** Twenty-five adult male Wistar rats weighing between 180 and 200g were randomly assigned to five groups of five rats each. Group I was the unexposed control group, while Group II was the Cypermethrin (10mg/kg/bw) exposed group. Groups III-V were exposed to Cypermethrin at standard doses of 10 mg/kg/bw and orally administered with ascorbic acid (5000mg/kg/bw), Alpha-tocopherol (3000mg/kg/bw), and coadministration of Ascorbic acid and Alpha-tocopherol respectively for 28 days. At the end of the experiment, the rats were euthanized, and their testes were harvested and processed for biochemical and histopathological analysis. **Results:** The findings revealed elevated malondialdehyde (MDA) levels, as well as a significant decrease in superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) activities ($p<0.05$). There were no visible histological changes in the testes, and co-administration of ascorbic acid and alpha-tocopherol restored biochemical parameters better than single administration. **Conclusion:** Coadministration of ascorbic acid and alpha-tocopherol reduces cypermethrin-induced oxidative stress better than single administration, possibly due to their complementary antioxidant properties.

Keywords: *Cypermethrin, Testicular injury, Rats, Ascorbic acid, Alpha-tocopherol.*

INTRODUCTION

Pesticides are widely used to control agricultural pests and insects that pose public health risks[1]. There have been reports of insecticide toxicity outbreaks in animals and humans, usually as a result of direct insecticide exposure or indirectly through contaminated feeds or water[2]. In experimental animals,

chronic insecticide exposure causes chronic neurological syndrome, malignant tumours, immunosuppressive action, teratogenic effect, abortion, and decreased male fertility [3]. Pyrethroid is a class of pesticides [4]. Natural pyrethrins derived from the botanical insecticide pyrethrum, an extract obtained from the flowers of *Chrysanthemum cinerariaefolium*, are used to create synthetic pyrethroid pesticides [5]. Pyrethroids have emerged as the most effective global insecticides in a variety of applications due to their high insecticidal potential, rapid metabolism, and low tissue accumulation, among other benefits [6]. Additionally, it is increasingly replacing organophosphates and banned organochlorines in many applications [7]. However, pyrethroid insecticides are widely distributed and pollute the environment as a result of excessive and unregulated application [8]. Pyrethroid insecticides enter the environment via several routes, including inhalation of contaminated air and dust, ingestion of pesticide-contaminated food, and skin contact [2]. The pyrethroid class of organic pesticides, which were previously thought to be safe for household and industrial use, have been linked to reproductive toxicity [9,6]. According to Abdou [10], Cypermethrin, a type II pyrethroid, caused alteration in the histology of the testis ranging from seminiferous tubule atrophy and Germ cell disorganisation to Sertoli and Leydig cell atrophy. Additionally, pyrethroid-induced damage has resulted in significant decreases in testicular weight, sperm count, sperm motility, and abnormal sex hormone levels [11,12]. The adverse effects of pyrethroid on the testes have been linked to oxidative stress [13]. Under normal conditions, cells have non-enzymatic and enzymatic pathways that maintain cellular redox homeostasis [14]. Glutathione (GSH) and the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidases (GPx), and catalase (CAT) are examples [15]. When this homeostasis is disrupted, either by depletion of antioxidant molecules like GSH or inhibition of antioxidant enzymes, reactive oxygen species form, resulting in testicular cell dysfunction and disintegration [16]. Non-enzymatic antioxidants, which are usually derived from food, help to maintain cellular redox homeostasis. Water-soluble antioxidants like ascorbate and fat-soluble alpha-tocopherol are examples [17]. While ascorbate protects the hydrophilic and extracellular environment from oxidative damage [18], alpha-tocopherol is a major membrane protector against reactive oxygen species and lipid peroxidation [19]. Few studies have reported the combinatorial antioxidative potential of ascorbic acid and alpha-tocopherol [20,21]. Studies have shown that pesticide exposure is a proven factor in male reproductive system impairment and infertility. Researchers and the general public have grown increasingly concerned about the risks of pyrethroid exposure to male reproductive health, necessitating additional efforts to find solutions, thus necessitating this study, which aims to investigate the mitigating effect of ascorbic acid and alpha-tocopherol against Pyrethroid-induced toxicity.

MATERIAL AND METHODS

Experimental Animals

Twenty-five adult male Wistar rats weighing between 180g and 200g were obtained from the vivarium at the Faculty of Basic Medical Sciences, University of Medical Sciences Ondo. Before commencing the experiment, the rats were allowed to acclimatize for two weeks and allowed access to standard rat chaw obtained from Chiku Farms limited and water *ad libitum* and subjected to natural photoperiod of 12hr-light/dark cycle.

Ethical Consideration

The University of Medical Sciences Ondo's Ethical Research Committee granted ethical approval with the number NHREC/TR/UNIMED-HREC- Ondo St/22/06/21. We ensured that all experimental animals were handled following the National Health Research Ethics Committee's protocols and guidelines.

Chemicals

Cypermethrin

Commercially grade Cypermethrin-based pesticide with trade name Avestrin® 10% E.C, [(RS)-cyano-(3-phenoxyphenyl)methyl-(IRS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane carboxylate] manufactured by Harvestfield industries Ltd Lagos, Nigeria was used for this study.

Alpa-tocopherol

Alpa-tocopherol capsules manufactured by Olive Healthcare Dabhe, India, were purchased from Ever Destiny Pharmaceuticals Limited Lagos, Nigeria. Each soft gelatin capsule contains Vitamin E acetate USP 1000 IU.

Ascorbic acid

Vitamin C tablets produced by Chemo-Pharma Laboratories Limited, Lagos Nigeria with the NAFDAC registration number 04-3486 were purchased from Uche Care Pharmaceuticals Ondo City, Nigeria. Each tablet contains Ascorbic Acid B.P 100mg.

Experimental Design

Following the acclimatisation period, the rats were randomly divided into five groups, as follows:

Group I consist of the unexposed control rats administered with distilled water only.

Group II consists of the exposed untreated group orally administered with cypermethrin at a dosage of 10mg/kg/bw.

Group III was the cypermethrin-exposed group

administered with Ascorbic acid at a dosage of 5000mg/kg.

Group IV were the cypermethrin-exposed group administered with α-tocopherol at a dosage of 3000mg/kg.

Group V was the cypermethrin-exposed group co-administered with ascorbic acid and α-tocopherol at a standard dosage of 5000mg/kg and 3000mg/kg respectively.

The experiment lasted for 28 days. While Cypermethrin was administered twice weekly, ascorbic acid and α-tocopherol were administered daily by oral gavage.

Tissue Preparation

Following the experiment, all animals were euthanized directly via cervical dislocation, and the testes were harvested and immediately transferred into 10% neutral buffer formalin for histopathological analysis. For the biochemical analysis, the testes of rats in each group were rinsed in phosphate-buffered saline, transferred into freshly prepared PBS solution, and frozen at -70°C until analysis for oxidative stress parameters.

Biochemical Studies

Homogenate samples with a concentration of 0.2 g/ml obtained from the testes of the experimental rats were analysed for Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase activities. Briefly, for MDA estimation, a thiobarbituric acid reactive substances (TBARS) assay kit obtained from Randox laboratory United Kingdom was used to measure the lipid peroxidation product of MDA equivalent. One hundred microlitres (100 µl) of homogenate was mixed with 2.5 ml reaction buffer and heated at 95 °C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation product in gram per tissue MDA levels was expressed in terms of µmoles/mg and computed with a molar extinction coefficient of $1.56\times10^{5}M^{-1}Cm^{-1}$[22]. For Superoxide Dismutase (SOD) activity, 100µl of supernatant was diluted in 900µl of distilled water to make a 1-in-10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started with the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds and SOD activity was determined accordingly [23]. Glutathione Peroxidase (GPx) activity was estimated as described by Rahman et al., [24]. The reaction mixture containing final concentrations of 1.5mM tris HCl pH 7, 10% triton x-100 (peroxide free), 2Mm NADPH, 1.5mM GSH, 1Mm Glutathione Reductase, 5% NaN3, and 100µl cell lysate was initially pre-incubated at 37 ^{0}C. Initial absorbance was registered at 340nm for 5 minutes at intervals of 1.5 minutes. 10mM H_2O_2 was added and the final absorbance reading was also registered at the same wavelength for an additional 10min at intervals of 1.5min. GPx activity was calculated by the change in the absorbance value at 340nm and expressed as micromoles of NADPH/minute/µg protein or µmoles GSH consumed/min/mg protein. Catalase activity was estimated as described by Sinha, [25]. Briefly, 0.2 ml of the sample was mixed with 0.8 ml distilled H_2O to give 1 in 5 dilutions of the sample. The assay mixture contained 2ml of solution and 2.5ml of phosphate buffer (10 mM pH 7) in a 10ml flat bottom flask, then 0.5ml of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at 25°C. Finally, 1ml portion of the reaction mixture was withdrawn into 1ml dichromate/acetic acid reagent at 60-second intervals after which the hydrogen peroxide content of the sample was determined [25].

Histopathological Examination

Adequately fixed excised testes from the control and the experimental groups were processed as described by Bancroft *et al.* [26]. Briefly, the tested were temporarily fixed in 10% neutral buffered formalin for 24 hours before being transferred into Bouin's fluid for adequate fixation. The samples were dehydrated with grades of alcohol and cleared in two changes of xylene, followed by embedding in paraffin and sectioned at 4 µm. The sections obtained were then stained with Harris Hematoxylin and Eosin (H and E) for histopathological examinations and viewed using an Olympus microscope.

Statistical Analysis

The obtained data were expressed as mean standard deviation mean, with n=5. The statistical significance level of treatment effects was evaluated by one-way analysis of variance (ANOVA) using SPSS software (version 25.0 x64; SPSS Inc, Chicago USA). GraphPad Prism version 8.0.1 (244) was used to analyze all parameters and Post hoc comparisons between groups were obtained by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when the p-value was <0.05.

RESULTS

General physical observations in this study reveal that oral administration of cypermethrin at the dosage of 10mg/kg/BW resulted in a marked decrease in body weight of animals, loss of appetite and heightened aggressiveness in the rats. Although there were no apparent lesions or cytopathic effects in testicular

histology across all experimental groups, the findings revealed a significant oxidative stress inducement characterised by elevated MDA levels with a decline in SOD, GPx, and CAT values. Furthermore, there was a relative reversal of the oxidative imbalance in the vitamin-treated groups, possibly due to the ameliorating effects of these vitamins.

Histopathological studies

The histological results of this study revealed that the oral administration of cypermethrin at a dose of 10mg/kg/bw to adult male Wistar rats for 28 days showed no apparent cytopathic changes in the testes. The testes of the unexposed control rats revealed a normal seminiferous tubule with the lumen containing fully developed germinal cells with spermatozoa. The interstitial spaces contain Leydig cells, while the interstitial connective tissue appears normal (**Figure 1a**). While the Untreated cypermethrin-exposed rats' testes showed normal testicular histology with no visible lesion (**Figure 1b**), the histological examination of the Ascorbic Acid and Alpha-Tocopherol and the combinatorial administration of the vitamin-treated rats appeared normal with no visible lesion evidenced by a normal seminiferous tubule with spermatozoa in the lumen and fully developed germinal cells. Leydig cells are visible in the interstitial spaces, as are interstitial connective tissues (**Figures 1c, d, e**).

Biochemical studies

Table 1 shows the mean and standard deviation of Malondialdehyde (MDA) concentrations, Superoxide dismutase (SOD), Glutathione peroxidase (GPX), and Catalase (CAT) activities across all groups. The MDA concentrations in the cypermethrin-exposed untreated group increased significantly when compared with the unexposed control group and other treatment groups. Additionally, the coadministration of ascorbic acid and alpha-tocopherol showed significant improvement in the MDA level bringing it close to the control concentration values (**Figure 2a**). There was a significant reduction in superoxide dismutase (SOD) activity in the cypermethrin-exposed untreated rats when compared to the control and other treatment groups. However, the coadministrative treatment with ascorbic acid and alpha-tocopherol showed better restorations of SOD activity (**Figure 2b**). Exposure to cypermethrin reduced the glutathione peroxidase (Gpx) values in the exposed rats. However, the administration of ascorbic acid and alpha-tocopherol restores the GPX values in the treatment group with the most significant restoration occurring in the vitamin-coadministered group. **Figure 2d** indicated that the ascorbic acid and alpha-tocopherol- better restored the catalase activity when compared to the cypermethrin-exposed untreated rats.

DISCUSSION

Pyrethroids are valuable agricultural pest management tools with a variety of household applications for controlling insect pests and disease vectors worldwide [4]. Excessive and unregulated pesticide use can be toxic to the male reproductive system necessitating the current study which investigates the adverse effects of cypermethrin. The histological results of this study revealed that the oral administration of cypermethrin at a dose of 10mg/kg/bw for 28 days showed no apparent cytopathic changes in the testes. Testicular histology showed a normal seminiferous tubule with lumens containing matured spermatozoa, fully developed germ cells, Leydig cells, and Sertoli cells. There was no seminiferous tubule degeneration, Leydig cell degeneration, spermatogenesis arrest, or congested interstitial layer. Kouamo et al.[27] previously reported that orally administering 130mg/kg cypermethrin to quails for 6 weeks causes degeneration of seminiferous tubule connective tissue. Additionally, Abdel-Razak et al. [28] also documented that cypermethrin treatment of rats at a dose of 5mg/kg bw for 28 days causes testicular damage such as incomplete sperm formation, sperm capture, interstitial cell shrinkage, and rupture of the seminiferous tube basement membrane, which is at variance with our observation in this study. This could be attributed to some factors, such as cypermethrin dosage administration as well as the duration of exposure. Previous research has shown that the ability of Cypermethrin to induce testicular damage is heavily dependent on the dosage and duration of exposure to this chemical, with findings indicating that chronic exposure to cypermethrin-induced alterations in the histology of the testis ranging from seminiferous tubule atrophy, Germ cell disorganization, to atrophy in the Sertoli and Leydig cells [9,16]. However, our findings show that 28 days of oral cypermethrin exposure at 10mg/kg/bw disrupts oxidant-antioxidant homeostasis but is insufficient to cause visible histoarchitectural damage. Also, the Blood-Testicular Barrier (BTB) is another important factor that could be involved in the current study's histological findings. While complete loss of BTB function results in infertility, during normal spermatogenesis, this structure must transiently open and close to allow immature germ cells to cross into the immune-privileged environment [29]. The BTB protects developing cells from the immune system by preventing developing sperm membrane antigens from entering the bloodstream[30], implying that blood-testicular barrier protection may be responsible for the normalcy in histological findings observed in this study. The deleterious effects of pyrethroid such as cypermethrin on the testes have been linked to oxidative stress. Under normal conditions, cells have non-enzymatic and

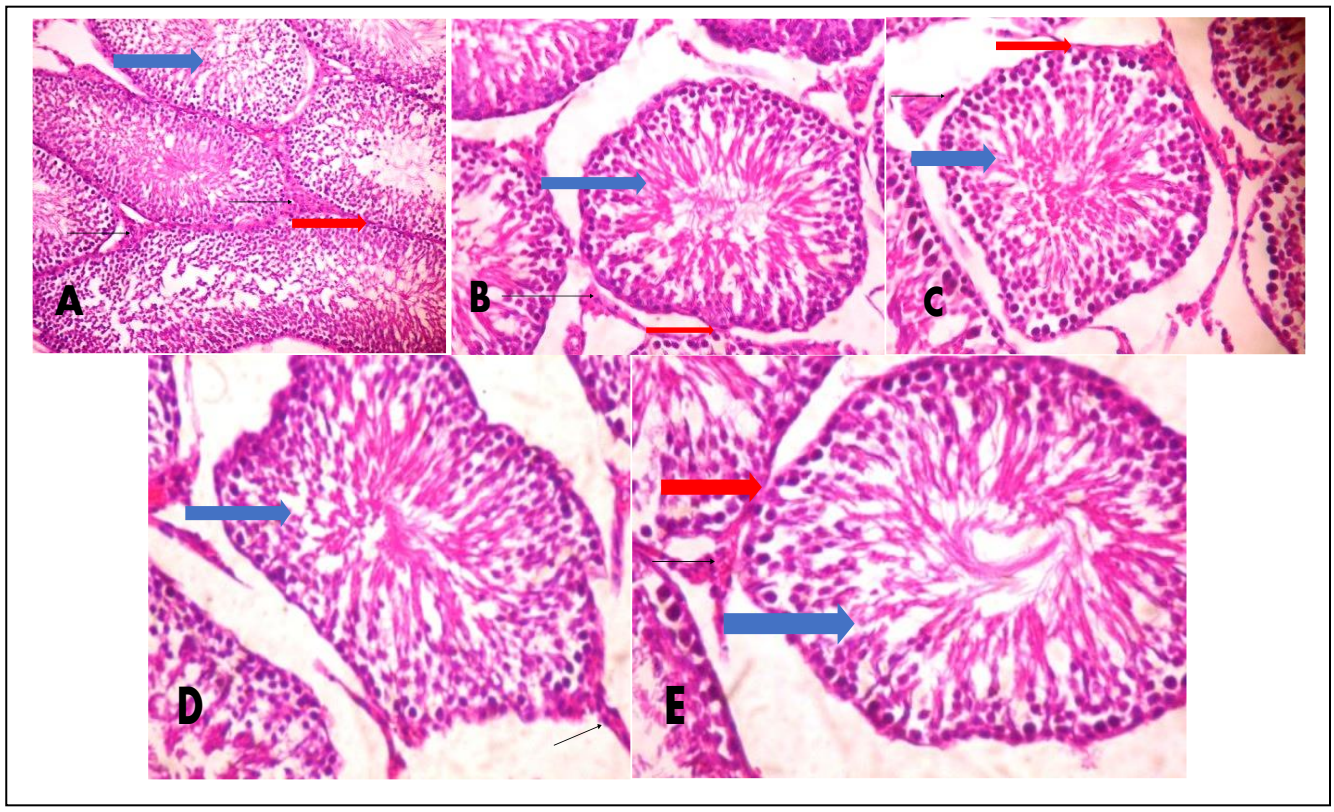

Figure 1. H&E-stained testicular histological sections. (**a).** The testes of the unexposed control rats show a normal seminiferous tubule with the lumen containing fully developed germinal cells with spermatozoa (blue arrow). The interstitial spaces contain Leydig cells (slender black arrows), while the interstitial connective tissue appears normal (red arrow) (mag =X100). (**b).** Untreated cypermethrin-exposed rats' testes show normal seminiferous tubules with lumen containing normal maturation stages with fully developed germinal cells and the presence of spermatozoa within their lumen (blue arrow), Leydig cells are also visible in the interstitial space (slender black arrow), as is interstitial connective tissue (red arrow) (mag= X400). (**c).** The testes of Cypermethrin-exposed rats administered with Ascorbic acid show a normal seminiferous tubule with spermatozoa in the lumen (blue arrow) and fully developed germinal cells. Leydig cells (slender black arrow) are visible in the interstitial spaces, as are interstitial connective tissues (red arrow) (mag= X400) (**d).** Cypermethrin exposed group administered with Alpha-tocopherol showed normal seminiferous tubules with lumen containing normal and completely developed germinal cells with spermatozoa evident (blue arrow). The interstitial spaces show Leydig cells (slender arrow) (mag= X400) (**e).** Co-administration with ascorbic acid and alpha-tocopherol showed normal seminiferous tubules with completely developed germinal cells. The interstitial spaces show Leydig cells (slender black arrow) and the interstitial connective tissue appears normal (red arrow) (mag= X400).

enzymatic pathways that ensure cellular redox homeostasis [14]. The disruption of this homeostasis by either the depletion of antioxidant molecules or the inhibition of antioxidant enzymes by overwhelming oxidants increases the formation of ROS, resulting in testicular cell dysfunction and disintegration [15]. In this study, the MDA levels in the untreated cypermethrin-exposed group were significantly higher than in the unexposed control group. This finding is consistent with the reports of Bouabdallah et al.[31] who documented that cypermethrin exposure, either alone or in combination with other pesticides, causes elevated MDA levels in the testes of rats. Malondialdehyde, a lipoperoxidation byproduct, causes membrane damage, disrupts cell structural integrity, and inactivates membrane-bounded enzymes[17]. As evidenced by an increase in MDA prooxidant levels in the testes, the production of free ROS in cypermethrin-exposed rats may have resulted in oxidative stress.

Oxidative stress disrupts the balance of free radicals and antioxidants, resulting in oxidative cellular injury. On the other hand, antioxidants prevent the overproduction of reactive oxygen species via enzymatic (superoxide dismutase, catalases, and peroxidases) and non-enzymatic mechanisms such as vitamins[13]. Lower MDA levels in cypermethrin-exposed rats demonstrated the mitigating effect of ascorbic acid and alpha-tocopherol when compared to exposed untreated rats.

Table 1. Mean and standard deviation of Malondialdehyde concentrations, Superoxide dismutase, Glutathione peroxidase, and Catalase activities across all groups.

PARAMETERS	EXPERIMENTAL GROUPS				
	CONTROL	CYP EXPOSED	CYP + ASCORBIC ACID	CYP + ALPHA-TOCOPHEROL	CYP + ASCORBIC ACID & ALPHA-TOCOPHEROL
MDA (µmoles/mg)	0.152±0.038^{b}	0.666±0.210^{a}	0.214±0.099^{b}	0.186±0.069^{b}	0.146±0.043^{b}
SOD (U/mg)	5.002±0.926^{a}	0.73±0.173^{d}	1.79±0.427^{c}	1.886±0.440^{c}	3.786±0.865^{b}
GPx (µmoles /mg)	39.044±4.195^{a}	20.708±1.497^{d}	27.044±1.521^{c}	26.93±2.134^{c}	33.298±2.744^{b}
Catalase (mmol/mg)	7.66±0.903^{a}	2.512±0.350^{c}	3.63±0.354^{b}	3.54±0.402^{b}	7.028±1.061^{a}

Key: *CYP: cypermethrin, VIT: vitamin, MDA: malondialdehyde, SOD: superoxide dismutase, GPX: glutathione peroxidase, CAT: catalase. Values are expressed as mean ± standard deviation (SD).* ^{a} *= 0.0000,* ^{b} *= 0.0001,* ^{c} *= 0.0163,* ^{d} *= 0.0353. There is a significant difference between the mean values of groups with similar letters at p<0.05. No significant difference at p<0.05 between the mean values of groups with dissimilar letters.*

Ascorbic acid can neutralise oxidative stress through an electron donation/transfer process and is a natural water-soluble antioxidant found in fresh fruits and vegetables. According to Caritá et al. [32], ascorbic acid can reduce unstable oxygen radical species while also regenerating other antioxidants such as alpha-tocopherol in the body. Furthermore, ascorbate has strong reducing properties due to the ease with which the moiety between its C2 and C3 carbons emits two protons and electrons, resulting in the formation of a dehydroascorbic acid (DHA) diketone group [33]. The hydroxyl groups at the double bond in the lactone ring donate protons and electrons, forming the diketone moiety of dehydroascorbic acid, which determines the strong reducing properties of ascorbic acid [34].

The most well-known and widely used antioxidant, on the other hand, is alpha-tocopherol, which protects cell membrane components such as polyunsaturated fatty acids (PUFA) and low-density lipoprotein (LDL) from oxidative damage caused by free radicals [35]. Tocopherols are structurally composed of a 6-chromanol group and an apolar phytal chain with the prefix α-, β-, γ-, or δ-, depending on the number and position of methyl groups attached to the chroman rings[36].

Tocopherols' antioxidant activity is conferred by the chroman head group. The chromanol ring, which has a -OH group, can donate a hydrogen atom to reduce free radicals and has a hydrophobic side chain that allows penetration into biological membranes. Furthermore, alpha-tocopherol inhibits lipid peroxidation by donating phenolic hydrogen to the ROO, resulting in tocopheroxyl radicals, cementing its antioxidative property even further[19]. According to the findings of this study, a comparative analysis of the effects of ascorbic acid and alpha-tocopherol administration revealed that treatment with co-administration of ascorbic acid and alpha-tocopherol resulted in a significant improvement in lowering the MDA level in the testes of the CYP exposed rats. This finding aligns with Zare et al. [20] who reported that combined administration of ascorbic acid and alpha-tocopherol provided greater protection against sodium metabisulphite-induced oxidative stress and reduced MDA levels in the exposed rats testes.

Superoxide dismutase is considered the primary antioxidant enzyme because it catalyses the dismutation of superoxide radicals to form hydrogen peroxide, providing the first line of defence against the harmful effects of reactive oxygen species in the cell. In this study, we observed a decrease in testicular SOD in cypermethrin-exposed rats. Studies by Akorede et al.[18] reported that carbamazepine pesticide significantly reduced testicular SOD enzyme levels. The decrease in SOD activity indicates decreased enzyme synthesis and increased metabolism or inactivation as a result of cypermethrin oxidative stress effects, which is evident in our study, such that the elevated MDA levels in the exposed rats may overwhelm and trigger SOD over-usage and subsequent rapid degradation. However, co-administration of ascorbic acid and alpha-tocopherol resulted in a significant increase in SOD activity when compared to the untreated cypermethrin group, a finding that supports Alkaladi,[37] who reported the elevated antioxidative properties of co-administration of ascorbic acid and alpha-tocopherol.

The lowered Glutathione peroxidase activity in the testicular tissues as seen in the cypermethrin group was in concord with the report of Abdel-razik et al. [28] who reported that treatment with 5mg/kg of cypermethrin five times a week for 28 days induced decreased glutathione peroxidase activity in rats testes. Glutathione peroxidase, an antioxidant enzyme that

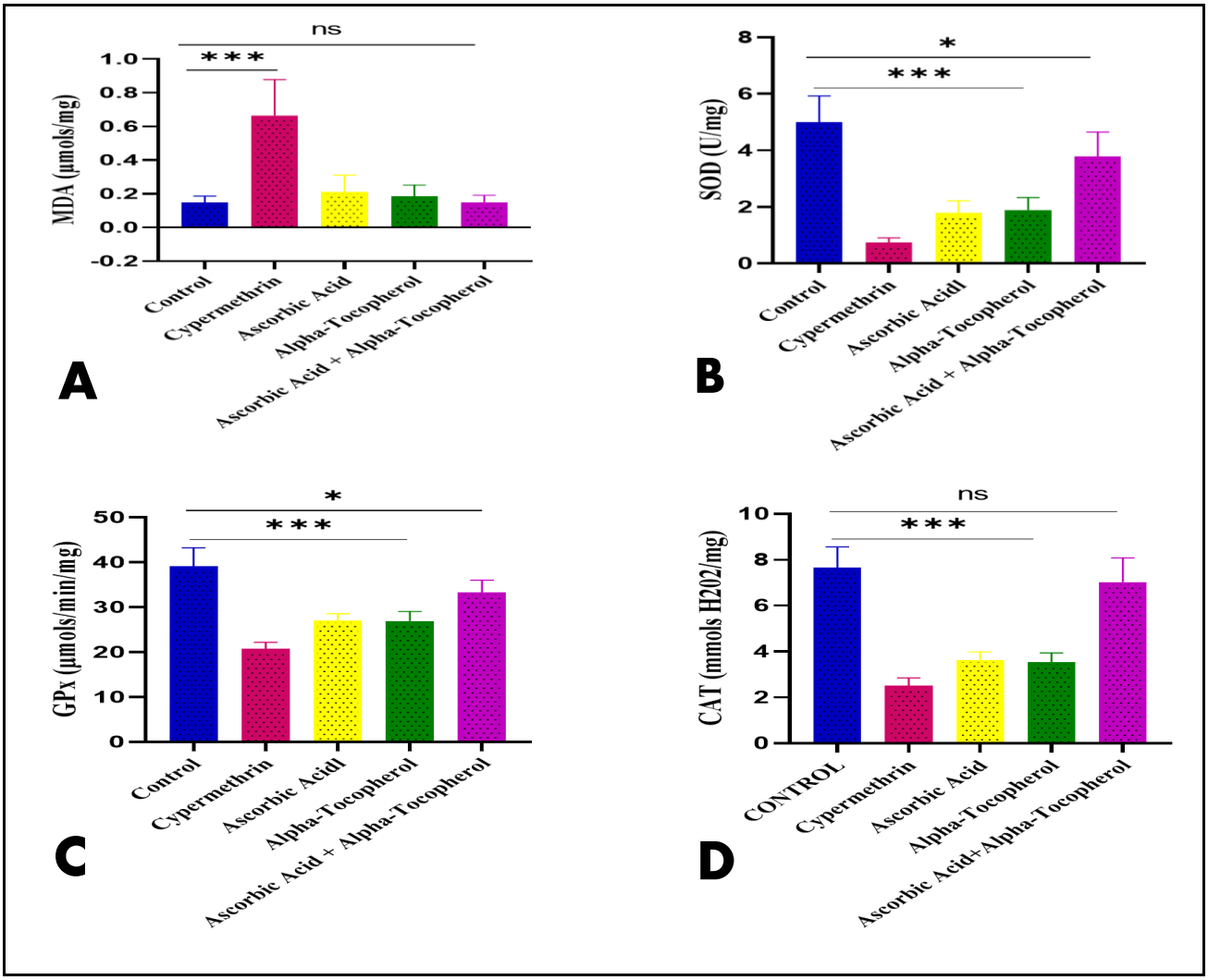

Figure 2. a. Effect of Ascorbic acid, Alpha-tocopherol and their co-administration on malondialdehyde (MDA) concentration in the testes of cypermethrin (CYP) exposed rats. **Narration**: *** = (significant difference in MDA concentration in the CYP exposed group when compared with the unexposed control). ns = 0.9878 (non-significant difference in MDA values in Ascorbic acid, Alpha-tocopherol, and ascorbic acid + alpha-tocopherol administered rats relative to the control). **b. Effect of Ascorbic acid, Alpha-tocopherol and their co-administration on superoxide dismutase (SOD) activity in the testes of CYP exposed rats**. **Narration**: * = 0.0454 (significant difference in SOD activity in ascorbic acid + alpha-tocopherol group. *** = 0.0010 significant difference in SOD activity in Ascorbic acid and Alpha-tocopherol administered groups relative to the control. **c. Effect of Ascorbic acid, Alpha-tocopherol and their co-administration on glutathione peroxidase (GPx) activity in the testes of CYP-exposed rats**. **Narration:** * = 0.0182 (significant difference in GPx activity in the ascorbic acid + alpha-tocopherol administered group relative to the control). *** = 0.0010 (significant difference in GPx activity in ascorbic acid and alpha-tocopherol administered groups relative to the unexposed control group). **d. Effect of ascorbic acid, alpha-tocopherol and their co-administration on Catalase (CAT) activity in the testes of CYP exposed rats. Narration:** *** = 0.0010 (significant difference in catalase activity in CYP ascorbic acid, and alpha-tocopherol groups in comparison to the control group). ns = 0.5959 (non-significant difference in catalase activity in ascorbic acid + alpha-tocopherol administered group when compared to control). Values are expressed as mean ± standard deviation (SD).

protects the cytoplasm and plasmalemma from lipid peroxidation by catalysing both the reduction of hydrogen peroxide and hydroperoxides, may have been depleted as a result of its response to cypermethrin-induced oxidative injury as observed in this. Treatment with ascorbic acid and alpha-tocopherol co-administration was found to restore GPX activity in the testes owing to their antioxidant property. Catalase is another antioxidant enzyme that plays an important role in the metabolism of a living system. It combats toxic

reactive oxygen species like superoxide and hydroxyl ions by converting them to water and oxygen and thus neutralising them.

The findings in this study revealed a significant decrease in catalase activity in the testes of cypermethrin-exposed rats. Reports by Simon et al. [11] documented Fipronil-induced oxidative damage in the liver of mice, as evidenced by a decrease in catalase activity. While the restoration of catalase activity following ascorbic acid and alpha-tocopherol administration demonstrated the antioxidant capacity of these non-enzymatic antioxidants, this assertion is consistent with Hidayatik et al.[21].

CONCLUSION

This study demonstrated that ascorbic acid and alpha-tocopherol can counteract the deleterious effects of cypermethrin. We also discovered that 10mg/kg/bw oral cypermethrin exposure disrupts oxidant-antioxidant homeostasis but is insufficient to cause visible histoarchitectural damage within the testes of exposed rats. Furthermore, we proposed that the inability to link oxidative stress to the histopathological effects of cypermethrin on the testes was most likely due to the blood testis barrier's protection and the short exposure duration. Long-term studies to determine the extent/severity of testicular damage that may result from long-term cypermethrin exposure at the administered dosage are recommended.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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