



Journal of Bioscience and Applied Research

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## Protective Effects of $\alpha$ -lipoic acid on Biological changes Induced by $\alpha$ -cypermethrin in Testis Rats

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### Abstract

$\alpha$ -cypermethrin is one of the most potent insecticide used worldwide. This study was planned to evaluate the possible role of  $\alpha$ -lipoic acid in  $\alpha$ -cypermethrin induced toxicity in rats. The treated groups were; the control,  $\alpha$ -cypermethrin,  $\alpha$ -lipoic acid and  $\alpha$ -cypermethrin and  $\alpha$ -lipoic acid groups. Our results showed that administration of  $\alpha$ -cypermethrin caused significant decrease in RBC count, PCV and Hb content and an increase of WBC count. Also,  $\alpha$ -cypermethrin caused significant increase in the levels of cholesterol, TGs, LDL-C and VLDL-C, while the HDL-C was decreased. In addition,  $\alpha$ -cypermethrin caused reduction in serum testosterone, FSH, and LH levels in intoxicated rats. Furthermore, the co-administration of  $\alpha$ -lipoic acid mitigated the toxicity of  $\alpha$ -cypermethrin by partially normalizing these biochemical parameters. Our results were supported by histopathological observations of testis. Our data suggest that  $\alpha$ -lipoic acid may have a protective role against  $\alpha$ -cypermethrin induced toxicity in rats.

Keywords:  $\alpha$ -cypermethrin, toxicity, blood, testis,  $\alpha$ -lipoic acid.

### Introduction

Pyrethrins are natural insecticides derived from chrysanthemum plant. Pyrethroids are synthetic derivatives that have greater chemical stability

than the natural Pyrethrins [1].  $\alpha$ -cypermethrin ( $\alpha$ -CYP) is one of the most potent and widely used type II pyrethroids and popular choice for pest

control. It is primarily absorbed by gastrointestinal tract as well as by inhalation of spray or only simply through skin contact [2].  $\alpha$ -CYP is

widely used. Therefore, it might present a risk to human health and environment [3]. There are extensive histomorphological, toxicological and biochemical reports on  $\alpha$ -CYP toxicity in different species of animals [4]. Hematological values are widely used to assess the general health condition of animals. It was found that  $\alpha$ -CYP caused anemia in treated animals which was indicated by decrease in RBC counts, Hb concentration and packed cell volume [5]. Infertility is one of the major health problems in life and approximately about 30% of this problem is due to male factors [6]. Infertility or sterility are often associated with low residual level of pesticides [7]. In animals, reproductive problems can be correlated with continues low-level exposure of pesticides [8]. It was found that  $\alpha$ -CYP can cause damage to male reproductive system, including testicular damage and alterations in sperm quality [9]. The testicular damage involved edema between seminiferous tubules, vacuolation and hyalinization in the tubules [10]. Environmental contaminants are known to cause oxidative stress, which may induce reproductive toxicity [11]. The mode of action of  $\alpha$ -CYP can be expected to have two ways: it may generate ROS that induce oxidative stress or it may accumulate in cell membrane and disturb membrane structure due to its hydrophobic nature [12]. Although testes have plenty of endogenous antioxidants for scavenging of free radicals generated, chronic exposure of  $\alpha$ -CYP

induced oxidative stress and caused excessive lipid peroxidation [13-14]. Antioxidants have the ability to protect the body against damage induced by oxidative stress [15]. Many studies have been done on the role of antioxidant substances that are taken into the body from diet [16]. Hence, there is a need for exogenous antioxidants to decrease oxidative stress induced by  $\alpha$ -CYP. Alpha lipoic acid (ALA) is a naturally occurring dithiol compound, which is synthesized enzymatically from octanoic acid in the mitochondria [17]. It is a compound found in food such as broccoli, spinach, beef, and meat [18]. ALA has an important role in mitochondrial energy metabolism [17]. Ideal antioxidant possesses many beneficial properties, including the ability to chelate metals, quench specific radicals and regenerate other antioxidants [18]. ALA is known to have protective effect against lipid peroxidation through its ability to bind to redox-active iron [19]. Therefore, the present study was conducted to investigate the possible protective effect of ALA on hematological and testicular toxicity induced by  $\alpha$ -CYP in rats.

## **Materials and Methods**

### **Chemicals**

$\alpha$ -CYP and ALA were obtained from Sigma-Aldrich, Germany. Reagent kit for determination of hemoglobin (Hb) was purchased from Biodiagnostics, Cairo, Egypt. Kit for determination of testosterone (T) was purchased from K-assay, WA, USA. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) kits were obtained from Biovender, Tokyo, Japan.

## Animals

Plastic cages were used in housing animals (5/cage). The animals were kept at 25°C, 60% humidity and 12 h light/12 h dark. They were allowed to acclimatize one week before the start of the experiment. Commercial rat chow and water were given to rats for feeding.

## Experimental Design

In this study, the experiment was carried out by using twenty male rats (*Rattus norvegicus*) which were randomly divided into four groups of five animals each. Experimental groups were designed as follows: The control group (GI): They received oral dose of saline followed by corn oil orally by gavage.  $\alpha$ -CYP group (GII); they received oral daily dose of  $\alpha$ -CYP (14.5mg/kg b.w.) dissolved in corn oil by gavage. ALA group (GIII); they received oral daily dose of ALA (20mg/kg b.w.) dissolved in saline by gavage.  $\alpha$ -CYP and ALA group (GIV); they receive the same oral daily doses of  $\alpha$ -CYP followed by ALA by gavage. The duration of the experiment was four weeks and the dose and route of administration of drug was chosen from previous study of Rotimil et al. (2015) [20] and we validated that in our previous work as well [21]. Animals were decapitated and blood was collected from trunk blood. The collected blood from each animal was divided into two tubes: the first one contains 10% EDTA for evaluation of hematological parameters and the second one was used for the separation of serum by centrifugation at 5000 rpm for 10 min for different blood analysis.

## Hematological Analysis

In the whole blood, Hb content was determined using commercial kit and PCV and RBCs count were determined according to their reported method [22].

## Biochemical Analysis

### *Determination of Serum Lipid Profile*

Total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C) were determined using standard kits (BIOMED-Diagnostics, Germany), (SPINREACT, Spain), and (BioSystems, Spain), respectively. Very low-density lipoprotein cholesterol (VLDL-C) and Low-density lipoprotein cholesterol (LDL-C) were calculated using Friedewal's formula [23], the units were expressed as mg/dl.

### *Hormonal Evaluation*

Testosterone, LH, and FSH hormones were estimated in serum using their commercial kits.

### *Determination of Sperm Quality*

The testis was minced in pre-warmed saline (37 °C) and the resulted suspension was used in the following analyses:

*Determination of Sperm Motility:* The motility of sperm was performed according to the method described by Morrisey et al., 1988 [24].

*Determination of Sperm Count:* The sperms were counted using a hemocytometer following the method of Freud and Carol (1964) [25].

*Determination of Sperm Abnormality:* Percentage of morphologically abnormal sperm was determined by the method described by Evans and

Maxwell (1987) [26]. Briefly, the percentage of live and dead spermatozoa was estimated by preparing a stained film using a drop from the sperm suspension with two drops from an

Eosin-nigrosin stain. The smears were made by placing a drop from the sperm suspension and one or two drops of the previously warmed (37 °C) eosin-nigrosin stain at one end of clean slide. The smears were allowed to dry in the air and then examined using a high power (100x) oil immersion objective. At least 200 sperm cells from different fields on the slide were examined to determine the live and dead spermatozoa. Normally live spermatozoa had completely unstained heads. Partially or completely red stained heads were classified as dead. Having calculated the dead/live ratio, the same stained slide was used to determine the percentage of abnormal spermatozoa. At least 200 sperm cells from different fields were examined and the number of abnormal ones was calculated as a percentage.

### **Histopathological Examination**

For preparation of samples used in the examination by light microscopy, specimens of testis were collected from all experimental groups and fixed in 10% neutral buffered formalin. The slides were stained with haematoxylin and eosin stain (H&E)[27]. Then sections were examined using light microscopy.

### **Statistical Analysis**

All variables were compared using one-way analysis of variance (ANOVA) followed by LSD multiple range test. Differences at  $P < 0.05$  were

considered significant. Statistical tests were performed using SAS statistical software (SAS v.9.2, SAS institute, inc).

## **Results**

### **Hematology**

Exposure to  $\alpha$ -CYP (G II) significantly lowers the values of Hb concentration, PCV and RBC count and increased WBC count value relative to values obtained for the control group. Exposure to  $\alpha$ -CYP followed by concomitant treatment with ALA significantly increases the values of Hb content, PCV and the RBC counts and decreased the WBC count value when compared with  $\alpha$ -CYP group. Treatment with only ALA did not significantly affect the hematological parameters when compared to the control group (Table 1).

### **Biochemistry**

In this study it was obvious that  $\alpha$ -CYP treatment significantly ( $P \leq 0.05$ ) increased the values of total cholesterol (TC), triglycerides (TGs), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) relative to the control group. Also, the results showed that high density lipoprotein-cholesterol (HDL-C) level in  $\alpha$ -CYP treated group was significantly ( $P \leq 0.05$ ) decreased relative to control rats (Table 2). Concomitant treatment with  $\alpha$ -CYP and ALA significantly ( $P \leq 0.05$ ) decreased the values of TC, TGs, LDL-C and VLDL-C compared to  $\alpha$ -CYP treated group (Table 2).

The results also showed that concomitant treatment with  $\alpha$ -CYP and ALA significantly

( $P \leq 0.05$ ) increased the HDL-C value compared to  $\alpha$ -CYP treated group. No significant ( $P \leq 0.05$ ) changes in the studied parameters were observed among ALA group when compared to the control (Table 2).

#### Biochemical Indicators of Testicular Function

Mean levels of serum testosterone, FSH and LH were significantly lower in the  $\alpha$ -CYP group relative to those obtained for the control group. On the other hand, their levels in the  $\alpha$ -CYP co-treated ALA group were significantly higher compared to the  $\alpha$ -CYP group. Treatment with

ALA did not affect significantly these parameters when compared with the control group (Table 3).

#### Sperm Quality

There was a significant decrease in sperm count and sperm motility while there was a significant increase in percentage of sperm abnormality in the rats treated with  $\alpha$ -CYP. In the  $\alpha$ -CYP co-treated ALA group, these altered parameters were ameliorated compared to the  $\alpha$ -CYP group. Treatment with ALA did not affect significantly these parameters when compared with the control group (Table 3).

**Table 1. Effect of ALA on some Hematological Parameters in Rats exposed to  $\alpha$ -CYP**

Group	Hb (g/dl)	PCV %	RBCs $10^6$ (cells/cmm)	WBCs $10^6$ (cells/cmm)
<b>I</b>	12.7 <sup>a</sup> $\pm$ 0.1	46.6 <sup>a</sup> $\pm$ 1.5	4.8 <sup>a</sup> $\pm$ 0.1	9474 <sup>a</sup> $\pm$ 51.7
<b>II</b>	8.7 <sup>b</sup> $\pm$ 0.1	29 <sup>c</sup> $\pm$ 0.7	3.5 <sup>c</sup> $\pm$ 0.1	12260 <sup>c</sup> $\pm$ 51
<b>III</b>	12.46 <sup>a</sup> $\pm$ 0.2	48.4 <sup>a</sup> $\pm$ 0.7	4.8 <sup>a</sup> $\pm$ 0.1	9472 <sup>a</sup> $\pm$ 76.5
<b>IV</b>	11.3 <sup>d</sup> $\pm$ 0.1	38.4 <sup>b</sup> $\pm$ 1.1	4.4 <sup>b</sup> $\pm$ 0.1	10072.4 <sup>b</sup> $\pm$ 57.04
<b>F (p)</b>	327.789* ( $<0.001^*$ )	71.915* ( $<0.001^*$ )	41.330* ( $<0.001^*$ )	487.561* ( $<0.001^*$ )

Normally distributed data was expressed in mean  $\pm$  SE and was compared using F test (ANOVA) and was using Post Hoc Test (LSD) for comparison between groups. The different superscripts are significant

\*: Statistically significant at  $P \leq 0.05$

**Table 2. Effect of ALA on Lipid Profile in Rats treated with  $\alpha$ -CYP.**

Group	TC (mg/dl)	TGs (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
<b>I</b>	116 <sup>a</sup> ± 1.4	83.4 <sup>a</sup> ± 0.9	45.2 <sup>a</sup> ± 1.6	48.6 <sup>a</sup> ± 1.1	22.2 <sup>a</sup> ± 1.2
<b>II</b>	193.6 <sup>c</sup> ± 1.3	112.8 <sup>b</sup> ± 3.5	30 <sup>c</sup> ± 0.7	124.7 <sup>c</sup> ± 1.1	38.9 <sup>c</sup> ± 0.3
<b>III</b>	115 <sup>a</sup> ± 0.8	84 <sup>a</sup> ± 1.2	46.4 <sup>a</sup> ± 1.2	45.8 <sup>a</sup> ± 1.4	23 <sup>a</sup> ± 0.2
<b>IV</b>	145.2 <sup>b</sup> ± 1.3	88.4 <sup>a</sup> ± 1.3	38.2 <sup>b</sup> ± 1.02	75.6 <sup>b</sup> ± 1	29.04 <sup>b</sup> ± 0.3
<b>F (p)</b>	905.998* (<0.001*)	48.744* (<0.001*)	41.211* (<0.001*)	1020.881* (<0.001*)	149.624* (<0.001*)

See footnote to Table 1.

**Table 3. Effect of  $\alpha$ -lipoic acid on testis hormones and sperm parameters of rats exposed to  $\alpha$ -cypermethrin**

Group	Testis					
	Hormones			Sperm parameters		
	T (pg/ml)	LH (ng/ml)	FSH (ng/ml)	Sperm counts million (n/ml)	Sperm Motility (%)	Sperm abnormality (%)
<b>I</b>	4.2 <sup>a</sup> ± 0.1	1.4 <sup>a</sup> ± 0.04	0.4 <sup>a</sup> ± 0.02	76.6 <sup>a</sup> ± 0.9	85.8 <sup>a</sup> ± 0.9	13 <sup>a</sup> ± 1.7
<b>II</b>	1.9 <sup>c</sup> ± 0.01	0.7 <sup>c</sup> ± 0.02	0.3 <sup>c</sup> ± 0.01	47.2 <sup>c</sup> ± 0.9	56 <sup>c</sup> ± 1.1	33 <sup>c</sup> ± 1
<b>III</b>	4.4 <sup>a</sup> ± 0.1	1.4 <sup>a</sup> ± 0.01	0.5 <sup>a</sup> ± 0.01	73.4 <sup>a</sup> ± 1.2	86.8 <sup>a</sup> ± 0.8	10.8 <sup>a</sup> ± 0.7
<b>IV</b>	3.5 <sup>b</sup> ± 0.1	1 <sup>b</sup> ± 0.01	0.4 <sup>b</sup> ± 0.01	55.4 <sup>b</sup> ± 1.2	73.8 <sup>b</sup> ± 1.2	20.4 <sup>b</sup> ± 1.7
<b>F (p)</b>	174.311* (<0.001*)	235.260* (<0.001*)	75.786* (<0.001*)	173.284* (<0.001*)	204.604* (<0.001*)	56.496* (<0.001*)

See footnote to Table 1.

### ***Histopathology of the testis***

Light micrographs of testis sections stained with H&E showed testicular tissue of the control group with typical architecture in which seminiferous tubules are separated by narrow interstitial tissue (Figure A-1) and high germinal epithelium with aggregation of spermatozoa in the lumen (Figure A-2). Testicular tissue of  $\alpha$ -CYP group showing distorted arrangement of seminiferous tubules with wide lytic interstitial tissue, congested blood vessel (Figure A-3) and necrosis and atrophy of germinal epithelium with spacious

free spermatozoa (Figure A-4). Testicular tissue of ALA group showed healthy seminiferous tubules in between well preserved interstitial tissue (Figure A-5). Spermatogenesis was shown with normal amount of spermatozoa (Figure A-6). Testicular tissue of  $\alpha$ -CYP and ALA group showed restoration of nearly normal seminiferous tubules with presence of normal interstitial tissue (Figure A-7) and germinal epithelium, which was more or less normal, and reappearance of spermatozoa (Figure A-8).

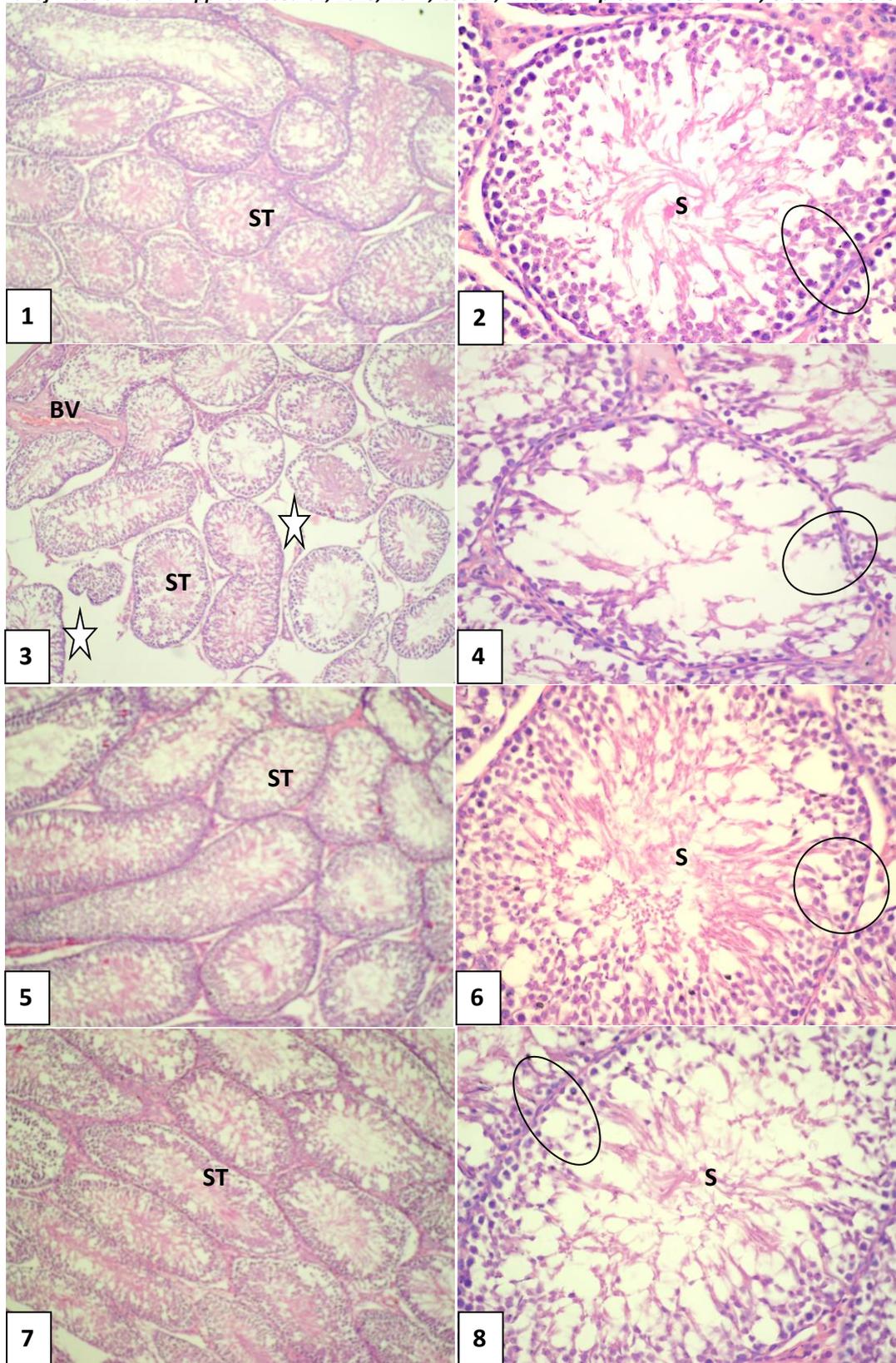


Figure A. Light micrographs of testis sections stained with H&E. (1) Testicular tissue of the control group showing typical architecture in which seminiferous tubules (ST) separated by narrow interstitial tissue. (2) Enlarged part from figure (1) with high germinal epithelium (circle) with aggregation of spermatozoa in the lumen (S). (3) Testicular tissue of  $\alpha$ -CYP group showing distorted arrangement of seminiferous tubules (ST) with wide lytic interstitial tissue (star) and congested blood vessel (BV). (4) Enlarged part from figure (3) where necrosis and atrophy of germinal epithelium (circle) with spacious free spermatozoa. (5) Testicular tissue of ALA group showing healthy seminiferous tubules (ST) inbetween well preserved interstitial tissue. (6) Enlarged part from figure (5) where spermatogenesis are shown (circle) with normal amount of spermatozoa. (7) Testicular tissue of  $\alpha$ -CYP and ALA group showing restoration of nearly normal seminiferous tubules (ST) with presence of normal interstitial tissue. (8) Enlarged part from figure (7) where germinal epithelium (circle) more or less normal and reappearance of spermatozoa (S).

## Discussion

Hematological parameters such as PCV and Hb concentration and are used to assess the oxygen carrying capacity and immunological condition of the bloodstream [28]. In the present study, there was a significant decrease in RBC count, PCV and Hb content and an increase of WBC count. Similar results were obtained by Nair et al.(2010) [29] and Orun et al. (2014) [30]. Decrease in hemoglobin content could possibly be due to suppression of erythropoiesis and heme synthesis and also to devastation of erythrocytes in hemopoietic tissue [31]. Activation of immune system of the body may result in an increase in release of WBC into the blood leading to an increase in number of WBC [32]. The altered lipid profile, demonstrated in this study, may be an indication that  $\alpha$ -CYP exposure may affect lipid metabolism. Similar results were obtained by Yousef et al. (2003) [32]. On the other hand, the improving effect of ALA on alteration of lipid profile, observed in this study, were reported by previous authors [33] in case of metabolic hepatosteatosis. The function and structure of the testicular epithelial tissue in humans and animals is adversely affected by natural and artificial environmental pollutants including pesticides [34]. Many studies showed the adverse effect of  $\alpha$ -CYP on the male reproductive system [35]. In the present study  $\alpha$ -CYP led to male reproductive toxicity indicated by reduction in serum testosterone, FSH, LH levels and alterations in sperm quality parameters (sperm count, sperm motility and sperm abnormality). Similar results were obtained by [36-38]. Synthetic pyrethroid

have been classified as endocrine-disrupting compounds as they possess hormonal activities [39]. Pyrethroids interfere with the synthesis of androgens in the testes [40] as testosterone hormone by causing decrease in testicular like 17 $\beta$ -hydroxysteroids dehydrogenase and glucose-6-phosphate dehydrogenase [41]. The ability of  $\alpha$ -CYP to reduce testosterone biosynthesis may induce suppression of spermatogenesis, low sperm production and degeneration of germinal epithelium [40]. Spermatogenesis suppression may also be due to low FSH and LH levels, which are required for normal spermatogenesis [13]. Point mutation in germ cell may be the responsible for induced sperm shape abnormalities [42]. Also, there is a high amount of unsaturated fatty acids in the sperm membrane which are susceptible to lipid peroxidation which may damage the membrane and caused impaired sperm motility [43]. Decrease in sperm count could be a result of alkylation of thiol group in the sperm nucleus and tail or DNA damage in the testis [44]. Endogenous antioxidants system may not be able to alleviate or fight degenerate diseases hence need for medical plants to maintain optimal cellular function [45]. Many previous studies have supported a positive role of plants that have antioxidant activity to protect or prevent the side effect of many xenobiotics. ROS have special affinity for lipids, proteins, carbohydrates and nucleic acids [46] thus threatening the integrity of these various biomolecules [47]. Mammalian cells are equipped with both enzymatic and non-enzymatic antioxidants defenses with different

efficacies to cope with ROS and other free radical [48]. The antioxidant enzymes include SOD, CAT and GP<sub>x</sub>. Their role as protective enzymes is well-known and has been investigated extensively [49]. Oxidative stress defines an imbalance between the formation of ROS and anti-oxidative defense mechanisms. In oxidative stress, lipid peroxidation occurred due to excessive free radical production and is considered a primary mechanism of cell membrane destruction and cell damage [50]. Damage of tissue induced by generated ROS has been reported to be contributing factor in a variety of human disease including male infertility [51]. Although testes have plenty of endogenous antioxidants for scavenging of free radicals generated, chronic exposure of xenobiotics may cause oxidative injury [52]. It is well known that  $\alpha$ -CYP caused decrease in CAT, SOD and GP<sub>x</sub> and increase in lipid peroxidation in blood and testis of treated rats [1,37,53]. Accumulation of  $\alpha$ -CYP in testis and other reproductive organs may have accelerated oxidative stress leading to decreased viability of all types of cells in testicular tissue. This accelerated death of spermatogenic cells which was associated with abnormalities in sperm structure and function [54]. Also,  $\alpha$ -CYP exposure increased lipid peroxidation level in testis causing a membrane degeneration [55] which may lead to sperm alteration. The biochemical changes observed in the present study were supported by histological observations. The pathological changes induced by  $\alpha$ -CYP in the testis of treated rats are in

agreement with those observed by [40, 56-57]. Human may need for exogenous antioxidants to alleviate or fight degenerate diseases caused by oxidative stress. Some studies have been conducted on the protective effect of ALA against xenobiotic inducing testicular toxicity. In the present study, ALA increased T, LH and FSH levels associated with an improvement in sperm quality. Also, it alleviate histopathological changes induced by  $\alpha$ -CYP exposure. Similar protective effect of ALA was reported where ALA showed protective effect against disease caused by oxidative stress [53]. It has been reported that ALA, as a biological antioxidant, has a protective role against oxidative stress in blood and hematological changes induced by aluminium [58] against reproductive toxicity in rats treated with  $\alpha$ -CYP [50], chlorpyrifos [59] or polychlorobiphenyl [60]. According to Jedlineska et al. (2007), the stability of testicular blood barrier may be increased by the intake of antioxidants [61].

### Conclusions

The present study proved that  $\alpha$ -cypermethrin developed hematological and testicular toxicity in rats.  $\alpha$ -lipoic acid has the ability to alleviate these hematological and testicular alterations induced by  $\alpha$ -cypermethrin in treated rats. So, this study showed that care must be taken as regards the use of  $\alpha$ -cypermethrin insecticide in agriculture, house and veterinary purposes. Also, it emphasized the impacts of  $\alpha$ -lipoic acid supplementation, as exogenous antioxidant, on protecting persons exposed to  $\alpha$ -cypermethrin.

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