The study of the protective effect of vitamin E against cypermethrin toxicity on testicular histology in mice

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Abstract: Cypermethrin is pyrethroid insecticide that is used worldwide for pest control in agriculture and household use. Vitamin E is a potential antioxidant protecting cells from oxidative stress. The aim of the present study was to investigate the protective effect of vitamin E on cypermethrin-induced changes in mice testis. Forty adult male albino mice,were divided into four groups: group I: served as control given corn oil; group II: received cypermethrin (2.8 mg/kg b.wt.) in corn oil; group III:received vitamin E (100 mg/kg b.wt.); group IV:received both cypermethrin and vitamin E. All treatments were given for 14 days.Light and electron microscopic demonstrated that cypermethrin induced atrophic changes in Leydig cells, thickening of seminferous tubule basement membrane with increased electron density of spermatogonia nuclei and cytoplasm. Sertoli cells vacuolation and appearance of abnormal spermatids were demonstrated. In vitamin E treated group normal testicular parenchyma was observed. In contrast, vitamin E given with cypermethrin result in significant improvement in tubules and Leydig cells and amolerated cypermethrin toxicity. Conclusion: showed that administration of vit E can protect against.The results cypermethrin induced oxidative damage in mice testicular tissue.
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1. Introduction

The synthetic pyrethroids constitute a unique group of insecticides having pyrethrin-like structures with better performance characteristics and account for over 30% of insecticide used globally (Vijverberg and Van den Bercken, 1982; Soderlund and Bloomquist, 1989). Cypermethrin, a synthetic pyrethroid insecticide has been extensively used in the last two decades in many of the developing countries, especially in Saudi Arabia and Egypt, for combating agricultural pests and insects of veterinary as well as human concern (Assayed *et al.,* 2010a). The synthetic pyrethroids insecticides are widely applied in view of the fact that they have shown to possess a high insecticidal activity as well as a broad spectrum of high initial toxic action on several types of pest (Assayed *et al.,* 2010b).

Accidental exposure with pyrethroids in human and animals result from advertent use. Populations at highest risk of high dose exposure are producers, hygienic and pesticide workers, and small farm owners applying cypermethrin for plant protection; low dose exposure originates mainly from the household application of insecticides, contaminated food and water (Gorell *et al.,* 1999). In mammals, cypermethrin can accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, lung, blood, and heart (Hall *et al.,* 1980; Manna *et al.,* 2004).The toxicity of pyrethroid insecticides to mammals has received much attention in recent years because animals exposed to these insecticides showed changes in their physiological activities besides other pathological features (Sakr, 2003 and Glass, 2008).Pesticides may affect human reproduction by direct toxicity to the reproductive organs or by interference with hormonal function (Garcia, 1998). Recent studies have shown that a wide range of pesticides, in trace amounts, may lead to serious problems in both male and female animals, including infertility, increased mortality of offspring and behavioral changes such as aggression (Elbetieha *et al.,* 2001).Testicular tissues are most sensitive to environmental toxins (Hose and Guilletle, 1995).

Pyrethroids, a pesticide of dltamtherin led to increased frequency of abnormal sperm cells (Bhunya and Pati, 1990). Exposing of rats to a high dose of pyrethroids was reported to cause testicular changes including atrophy of seminiferous tubules, decomposition of sperms and interstitial edema (Mishra *et al.,* 1993). Previous studies on pesticides showed that their toxicity is linked to different mechanisms, including reactive oxygen species (ROS) generation and oxidative stress (Gupta *et al.,* 1999). Free radicals play an important role in toxicity of pesticides and environmental chemicals, pesticides may induce oxidative stress leading to generation, of free radicals and alterations in antioxidant status or the oxygen free-radical (OFR)-scavenging enzyme system (Assayed *et al.,* 2010a). Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity (Almeida *et al.,* 1997; Lopez *et al.,* 2007; Mansour and Mossaa, 2009). During the past few years, estimation of free radical generation and antioxidants defense has become an

important aspect of investigation in mammals (Zini *et al.,* 1993; Yousef, 2010). Our the present work recent studies were was carried out to evaluate the potential role of antioxidant, such as, vitamin C, vitamin E, b-carotene, isoflavones, folic acid, propolis, curcumin and grape seed proanthocyanidin extract for the protection of cells against oxidative damage due to pesticides, heavy metals and chemotherapeutic agent toxicities (Oda and El-Maddawy, 2012). Antioxidants can protect against the damaging effect of oxygen species on sperm quality (Yousef, 2010). The production of reactive oxygen species (ROS) is a normal physiological event in various organs including the testis. Overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Akiyama, 1999). Vitamin E is a potential antioxidant, and a lipid soluble vitamin present in biological membranes. High levels of vitamin E are found in selected mammalian tissues including testes (Acharya *et al.,* 2004).Vitamin E protects critical cellular structures against damage caused by oxygen-free radicals and reactive products of lipid peroxidation (Yousef, 2010).

Studies carried out with antioxidant such as α- tocopherol, have shown that they inhibit free radical formation (Kalender *et al.,* 2004; Kalender *et al.,* 2005).Vitamin E is believed to be the primary components of the antioxidant system of the spermatozoa (Surai *et al.,* 1998). It has been reported that lipid peroxidation was prevented by vitamin E (Meydani, 1995; Yousef *et al.,* 2006). Yousef (2010) reported that the ameliorating effect of VE against the toxicity of lambda-cyhalothrin, synthetic pyrethroids type II on semen quality may be due to their role as antioxidant through the reduction in LPO potential. The improvement in the histological structure of testes, epididymes and accessory sex glands was correlated to the potential role of VE and Se in scavenging ROS generated by dltamtherin (Oda and El-Maddawy, 2012).

The aim of the present study is to examine the environment hazards caused by pollutants used in agriculture and public health, particularly insecticide cypermethrin on the histology male mice testis and the possible role of antioxidants such as vitamin E in mitigating the negative effects of this toxic pesticide.

2. Material& Methods

2.1.Material

2.1.1.Animals

In this study 40 adult male albino mice (MF1), obtained from King Fahd Medical Research Center, King Abdulaziz University in Jeddah, were used. The animals were in good health having average weights (33± 2 g). The mice were transfered to biology lab faculty of science KAU and left one week for acclimatization at a temperature of (25± 2 g) under two systems of lighting (12L - 12D), white fluroscent and red light, and provided with standared mice pellets and water add labitum.

2.1.2. Cypermethrin

The chemical pyrethroid was obtained from one of the stores in Jeddah and imported by the Saudi Company, Delta chemical industries.

2.1.3. Vitamin E

It is a semi- liquid substance, stored in dark glass bottle because it is sensitive to light and under 2-8°C. It was obtained from Ballna Trading Co. Ltd. In Jeddah.

2.2. Experimental design

Mice were divided into four groups (each 10 animals) as follows: Group I: control, which was fed corn oil only throughout the experiment; Group II: cypermethrin group (CYP): which was fed 2.8 mg/kg body weigth of cypermethrin dissolved in corn oil orally through a feeding tube (Coombs *et al.,* 1976). GIII: vitamin E group: in which the animals were fed vitamin E (100 mg/kg b.wt.) in corn oil orally (Weber *et al.,* 1997). GroupVI: animals given vitamin E with cypermethrin (CYP+Vit.E), at dose of 100 mg/kg b.wt. vitamin E and 2.8 mg/kg cypermethrin dissolved in corn oil, orally throughout duration of experiment.

2.3. Histopathology

At the end of the experiment (14 days) the animals were anesthetized using ether, on an anatomy plate and the skin was removed and the testis were extracted by the application of the experimental technigue of tissue sectors. Sections of the testis were prepared for examination under the light and electron microscopy for identification of any histopathological changes. The methods followed for preparation of the sections were as follows:

For the histopathological examination, the testis were collected and fixed in a neutral buffered-fromalin. After a proper fixation for 48h, the tissue were cut into thinner pieces (2-3 mm thick). The samples were embedded in paraffin blocks. Sections of about 5µm were cut, stained with hematoxylin and eosin by the standard method, and examined under light microscope (Luna, 1968). The sections were viewed and photographed on a olympus light microscope (Olympus BX51, Tokyo, Japan).

For electron microscopic examinations of testis tissues, primary fixation was done in 3% glutaraldehyde in sodium phosphate buffer (200 mM, pH 7.2) for 3 h at 4 ◦C. Testis tissues were washed with the same buffer and postfixed in 1% osmium tetroxide in sodium phosphate buffer, pH 7.2, for 1 h at 4 ◦C. Tissue samples were washed with the same buffer for 3 h at 4 ◦C and then embedded in Araldite. Thin sections were cut with a Leica EM UC6 (Leica Co., Austria) ultramicrotome. Samples were stained with 2% uranyl acetate and lead citrate. The sections

were viewed and photographed on a Jeol 100 CXII transmission electron microscope (TEM) (Jeol Ltd., Japan) at 80 kV.

3. Results

3.1. Structure of control mice testis:

Control mice testis used in this study has normal features described in previous literature. Seminiferous tubules showed all stages of spermatogenesis starting from basal spermatogonia to top spermatid and mature sperms. Leydig cell clusters were seen between the tubules.Fig (1A), showed the normal electron microscopic structure of basal spermatogonia and nearby Sertoli cells (Fig 2 A).

3.2. Structure of testis of mice testis treated by cypermethrin:

(Fig 1B) Cypermethrin was found to affect developing germ cells sparing mature sperms. There was degenerative changes and vacuolation in the early stages of germ cell layers. Interstitial tissue between the tubules showed edematous changes and atrophy of Leydig cells (Fig1B). Electron microscopy showed thickening of tubule basal lamina, apoptotic changes in basal germ cells where their nuclei appeared highly electron dense. Sertoli cells contain electron dense residual bodies (Fig 2B).

3.3. Structure of testis of mice testis treated by Vitamin E group:

(Fig 1C) Shows normal tubules that contain all stages of germ cells especially spermatozoa. It is also clear that leydig cells between the tubules appear normal together with the thin wall blood capillaries. Electron microscopy also showed normal mature heads of spermatozoa. Note the presence of the spermatids with its acrosomal cap, residual bodies and some tails of the spermatozoa (Fig 2C).

3.4. Structure of testis of mice testis treated by cypermethrin+vitamin E group:

Fig (1D) showed seminiferous tubules with normal outlines ordinary layers containing almost all phasesof germ cells, including the spermatocytes and between the tubules there are leydig cells with normal sizes and thin wall blood capillaries, while under the electron microscope (Fig.2D) all components, the spermatocyte, the spermatozoa, the spermatogonia and the spermatidecan be detected.An increase in the number of the interstitial cells which contain large numbers of cell organelles such as mitochondria and rough endoplasmic reticulum can be also observed.

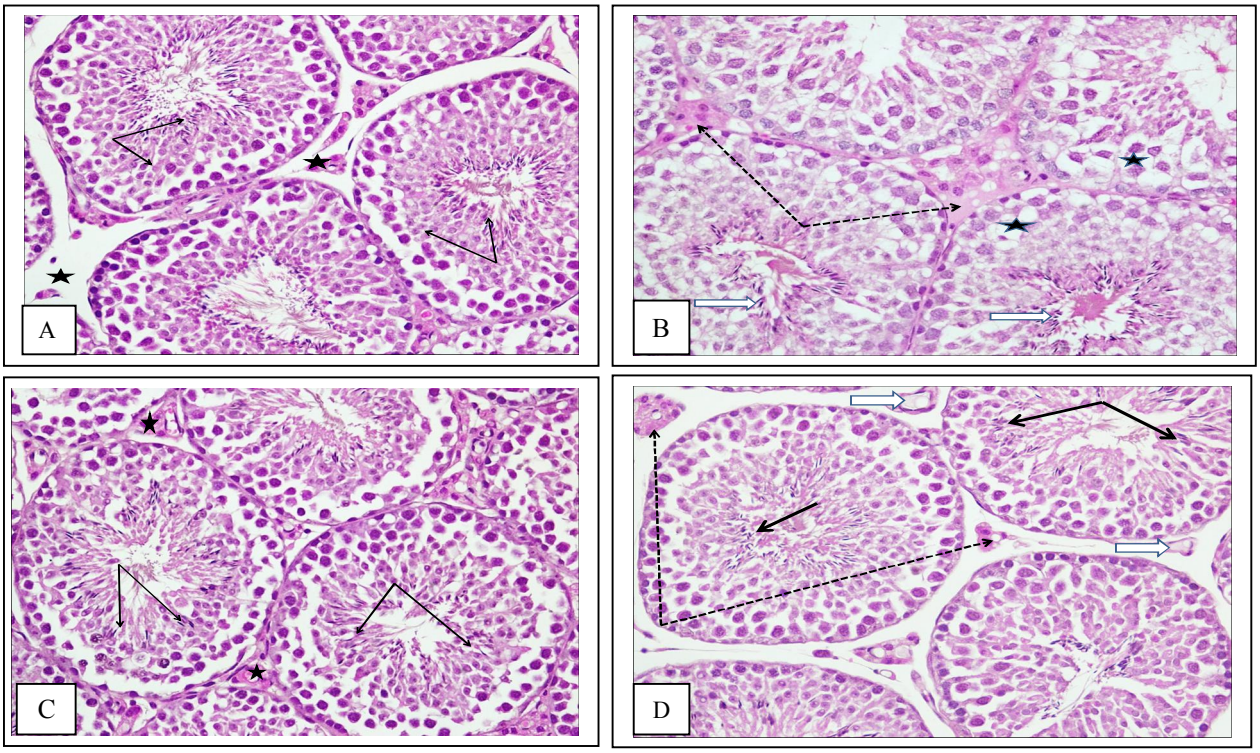

Fig 1.(A): The testicular section of the control group illustrates the natural form of the tubules and the presence of all stages of the germ cell layers including spermatozoa (black arrows) and leydig cells (star*). (B) Cypermethrin group: showingan affected primary germ cells (star *) while the tubules still containing spermatozoa. Also notice the presence of secretions in some tubules (white arrows) and interstitial edemawith atrophy of leydig cells (dotted arrows). (C) Vitamin E group: the seminiferous tubules are normal and contain all stages of germ cells especially spermatozoa that appear in the image as dark warheads with curly tails (black arrows). It is also clear that Leydig cells between the tubules are normal together with the thin wall blood capillaries (star *). (D) The cypermethrin + vitamin E group: the tubules are normal with regular

outlines, basal membraneis normal and enclosing all classes of germ cells, mature spermatocyte (black arrows) Leydig cells with normal sizes (dotted arrows)are seen between the tubules.Blood capillaries have thin walls (white arrows).
(A) X 400, (B) X100, (C) X100, (D)X400.

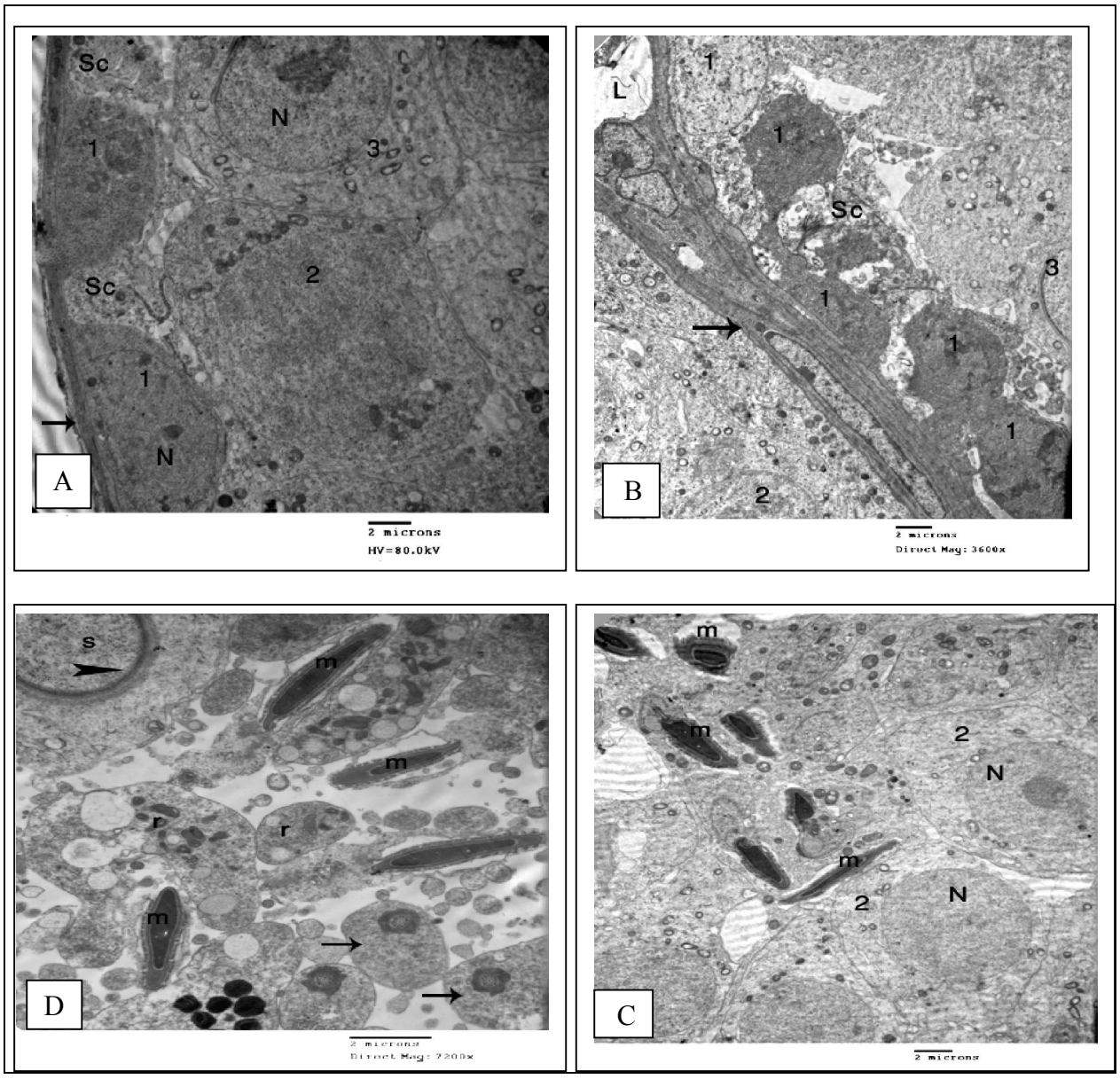

Fig. (2): Represents sections of the mice testis tissues under the electron microscope. (A) Control group:showing seminiferous tubules with normal spermatogonia(1), spermatocytes (2), spermatid (3) and Sertoli cells (Sc). (B) Testis of the cypermethrin group: showing thickening and basement membrane (arrows), large numbers of shrunken spermatogoni(1). They showed increase density of both cytoplasm and nucleus.Part of degenerated spermatocytes is seen phagocytized by Sertoli cells (Sc). (C): Vitamin E group showing normal mature heads of spermatozoa (m) near the lumen of the seminephrous tubules. Note the presence of the spermatide cells (s) with its acrosomal cap (arrow heads), residual bodies (r), and some tails of the spermatozoa (arrow). (D): Cypermethrin + vitamin E group:showing preservation of normal structure of most germ cell layers. Spermatocytes showed normal appearance (2), with normal the nucleus (N) which contains large nucleolus.Many normal spermatozoa are also seen (m). (A) X3600, (B) X3600, (C) X7200, (D) X3600.

4. Discussion

In the present study testicular toxicity of cypermethrin were assessed using both light and electron microscopy. The possible protective role of vitamin E against such toxicity was also evaluated.

Microscopic examination showed that cypermethrin has affected the testis tissues of the animals

particularly the initial germ cells. By electron microscopy there was thickening of basement membrane of seminiferous tubules besides deformation of spermatid stages and abnormalities in the shape and size of the spermatozoa. Thickening of basement membrane was described in literature in many cases of testicular toxicity due to drug or chemical exposure (El-Demerdash *et al.,* 2008; Wang *et al*., 2009) and could play a role germ cell atrophy and death related to interference with germ cell nutrition. Atrophy of Leydig cells observed in the present study could be due to vascular congestion and edema in its surrounding environment. The toxic compounds have been reported to cause adverse effects on reproductive functions and caused several pathological changes the testicular tissue of many vertebrates (Thea *et al.,* 2006). Pant *et al.* (1995) had previously reported that insecticides belonging to different groups such as organophosphorous malathion and bastocarbamate carbaryl or lannate were extensively studied in rats, mice and rabbits. Their toxic effect affectmany organs including testis where in the latter causing reproductive dysfunction represented in testicular atrophy and a decrease in sperm tubule diameter.

Bhunya and Pati(1990) demonstrated that the treatment of mouse with the industrial pyrethroid which is an deltamethrin insecticide had led to increased frequency of abnormal sperm cells which were similar to what observed by electron microscopy in the present study. In a more recent study done by Rodriguez *et al.* (2009) reported that feeding rats for 45 days with food containing doses of cypermethrin caused an impact on the sperm glands of the animals and led to changes in their reproduction.

The exposure of mice to poisonous materials was reported to cause damage to the testicular tissue and lead to decline in the diameter of the spermatozoa tubes and reduce the number and composition of the spermatocytes (Debnath and Mandal, 2000).

In the present study it was found that supplementation of Vitamin E lead to protection of mice testis against cypermethin induced testicular damage which that demonstrated the role of vitamin E as an antioxidant agent.Under the light microscope testis of group cypermethin + vitamin E showed normal sperm tubules and all layers of germ cell including spermatocytes.Leydig cells and surrounding blood capillaries showed also normal appearance.The electron microscopic examination revealed the presence of normal spermatocyte with many of spermatogonia,spermatida and spermatozoa.It is well known that vitamin E is a lipid – soluble vitamin that is present in biological membranes (Senthilkumar *et al.,* 2004) and it prevents oxidative damage by blocking the oxidation of polyunsaturated fatly acids (Trader and Packer, 1995; Aldana*et al.,* 2001). It efficiently protects against lipid peroxidation through its chain-breaking antioxidative activity (Serbecic and Beutelspaher, 2005), wherein vitamin E is converted to a weak free radical (the a-tocopherol radical) (Zaken *et al.,* 2001).

Vitamin E was reported to reduces the toxicity of the pesticide dichlorvos on the liver tissue, but does not protect it (Ogutcu *et al.,* 2008) and it prevents excess oxidative stress caused by the pesticide diazinon on the liver tissue (EL-Shenawy *et al.,* 2010). Vitamin E also has a protective role protecting the mice testis against the toxicity of cadmium (Ognjanovic *et al.,* 2010). Vitamin E reduced the toxicity of the insecticide lambda – cyhalothrin by increasing the proportion of the hormone testosterone and semen body weight and testis (Yousif, 2010).Also vitamin E reduced the toxicity of dichlorvos on the composition of the testis tissue the male rats (Dirican and Kalender, 2012) and reduced the toxicity of the pesticide deltamethrin on the reproductive system in male mice (Oda and El-Maddawy, 2012). The role of vitamin E in the improvement of the testicular tissue is marked and clear in the presence of normal and natural leydig cells and sertoli cells (Ozmen and Mor, 2012).As a summary it could be concluded that exposing the mice to the pesticide cypermethrin had caused changes in the histology of the testis leading to atrophy and damage of germ cells and intervening Sertoli cells. Abnormal spermatid and sperms were observed. both by light and electron microscopy. In addition the results of the current study showed that vitamin E has a protective role against the toxicity of the pesticide cypermentrin as it worked as an antioxidant reconfiguring the structures of the cells through its contribution in maintaining the balance between the formation of free radicals that result from toxic substance and the stimulation of antioxidant enzymes which restore the function and structure to normal.

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