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# Potential Protective Effect of Vitamin E against Reproductive Toxicity Induced by Cypermethrin in Male Rats

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## Abstract

This study was performed to examine the possible protective role of vitamin E against reproductive toxicity of insecticide cypermethrin (CYM) in male rats. Twenty five sexually mature male rats were randomized into 5 groups: group one was kept as a negative control. In the remaining 4 groups, CYM was orally administered at doses of 15 and 7.5 mg $kg^{-1}$(corresponding to 1/10 and 1/20 of $LD_{50}$) alone and / or in combination with vitamin E for 65 successive days. Blood samples were withdrawn for estimation of testosterone, FSH and LH in the serum. Semen analysis, sex organs weights, antioxidant enzymes superoxide dismutase (SOD); glutathione peroxidase (GPx) and catalase, (CAT) enzyme activities in testicular tissue and histopathological changes in testes were the parameters used in this study. The results showed that concurrent administration of vitamin E with CYM improved the relative weight of testes and increased sperm cell concentration and percentages of sperm motility and viability. There were also significant increases in serum testosterone, FSH and LH levels and in antioxidant enzymes activity in testes. This was associated with amelioration of testicular degenerative changes.In conclusion vitamin E exerts a protective role against testicular toxicity induced by CYM in male rats.

**Keywords:** Cypermethrin, Vitamin E, Male fertility, Sperms, Testosterone.

## Introduction

Infertility is one of the major health problems and about 30 % of this problem is due to male factors (Isidori *et al*., 2006). Several factors can interfere with the process of spermatogenesis and reduce sperm quantity and quality. Many diseases and conditions have deleterious effects on the process of spermatogenesis (Mosher and Pratt, 1991; Mahgoub and El-Medany, 2001; Agbaje et al., 2007 and Abdulbari *et al*., 2009).Moreover, more than 90% of male infertility cases are due to low sperm counts, poor sperm quality and both. The remaining cases of male infertility can be caused by a number of factors including anatomical problems, genetic defects (Liu *et al*., 2012) and hormonal imbalance (Shereen *et al*., 2013). Because of oxidative stress is evoked by many chemicals including some pesticides, the antioxidants can reduce the deleterious effects of insecticides and reduce the damaging effect of oxidative stress on testes (Shalaby *et al*., 2004 and Slaninova et *al*., 2009). Previous studies showed that Vitamin E could normalize the damaging effect of oxidative stress induced by oxygen free radicals (Shalaby *et al*., 2004 and Sanoka *et al*., 1997). intake of vitamins E and C can protect sperm DNA from oxidative stress in rat testes (Jedlinska *et al*., 2006) and antagonize testicular toxicity caused insecticide Lambda - cyhalothrin (Youssef, 2010). This study was performed to examine the possible protective effect of vitamin E against reproductive toxicity induced by cypermethirn (CYM) insecticide on male in rats.

## Material And Methods

**Drug:**

Cypermethrin was obtained from Kafer Elzayat (K.Z. Company) for Pesticides and Chemicals Company, Cairo, Egypt, as 20% Emulsifiable concentrate (EC) in the form of yellow liquid with specific odour. Vitamin E (Alpha-tocopherol) was obtained from Pharco Company for Pharmaceuticals, Alexandria, Egypt, in the form of soft gelatine capsules each containing 1000 mg of vitamin E.

**Animals and experimental design**

The experiment on rats was carried out in accordance with the recommendations of the National regulations and rules on animal welfare and Institutional Animal Ethical Committee (IAEC). Twenty five animals were subdivided into 5 groups of 5 animals. Group (1) was kept as control group negative (non-treated); groups (2) and (3) were orally given cypermethrin alone in a dose of 15 and 7.5 mg $kg^{-1}$ b.wt once daily for 65 consecutive days, respectively. Groups (4) and (5) were orally given cypermethrin in a dose of 15 and 7.5 mg kg-1 b.wt in combination with vitamin E (40

mg $kg^{-1}$ b.wt.) once daily for 65 consecutive days, respectively. Blood samples were collected and serum was separated for estimation of testosterone, FSH and LH level. Testosterone concentration was assayed using radioimmunoassay (RIA) method (Chen *et al.*, 1994) and LH and FSH concentrations were determined by ELISA technique (Ballester *et al.*, 2004).The rats of each group were sacrificed and the testes, prostate gland and seminal vesicles were dissected and weighed. Seminal contents of the epididymis were obtained after cutting the cuda epididymis and squeezing it on a clean sterile glass slide. The sperm progressive motility and concentration were determined. The percentage of sperm cell viability and morphological abnormalities were assessed using an Eosin - Nigrosin stain (Bearden and Fluquary 1980).Testicular tissue specimens were homogenized in 9 fold volumes phosphate buffered solution (PH 7.4). The homogenate was then centrifuged at 4000 rpm for 15 min at 40 $C^0$ and the supernatant was kept at -80 $C^0$ until used. Superoxide dismutase (SOD) activity was determined as described by (Nishikimi et al. 1972) while Glutathione peroxidase (GPx) and Catalase (CAT) activities were measured according to (Pagala and Valentine 1967) and (Sinha 1972), respectively. Samples from testes were collected from all experimental groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70-100%). Specimens were prepared using standard procedures for Hematoxylin and Eosin staining (Bancroft et al. 1996).

Data were expressed as means ± standard errors (S.E.) and were statistically analyzed using one-way analysis of variance (ANOVA). That followed by Turkey-Kramer's multiple comparison tests at significant level $P<0.05$ (Snedecor and Cochran 1986).

**Results:**

Oral administration of cypermethrin to male rats at a dose of 1/10 and 1/20 of $LD_{50}$ for 65 consecutive days caused significant decreases in the relative weight of testes, epididymis, seminal vesicles and prostate gland. Concurrent administration of cypermethrin (CYM) with vitamin E to male rats resulted in non-significant increase in relative weights of these sex organs when compared to the effect of cypermethrin alone (Table 1).

Semen analysis revealed that oral administration of CYM caused significant decrease in sperm cell concentration and percentage of sperm motility and viability (Table 2).There was also significant increase in sperm cell abnormalities. Co-administration of vitamin E with CYM significantly increased sperm cell concentration with non-significant increase in percentage of sperm motility and viability associated with non-significant decrease in sperm cell abnormalities.

**Table (1):** Effect of cypermethrin alone and/or in combination with vitamin E on relative weights of sexual organs of male rats. (Means ± S.E., n=5)

| Groups | Relative weight of sex organs (gm/100 gm b.wt.) | | | |
|---|---|---|---|---|
| | Testes | Epididymis | Prostate glands | Seminal vesicles |
| Negative control | 0.502±0.016$^a$ | 0.31±0.02$^a$ | 0.41±0. 06$^a$ | 0.472±0.01$^a$ |
| CYM (15 mg/kg ) | 0.302±0.02$^c$ | 0.206±0.01$^b$ | 0.24±0.01$^b$ | 0.32±0.01$^b$ |
| CYM (7.5 mg/kg ) | 0.304±0.01$^c$ | 0.210±0.01$^b$ | 0.26±0.01$^b$ | 0.31±0.01$^b$ |
| CYM (15 mg/kg ) + Vit. E (40 mg/kg) | 0.307±0.01$^c$ | 0.204±0.01$^b$ | 0.27±0.01$^b$ | 0.33±0.01$^b$ |
| CYM (7.5 mg/kg ) + Vit. E (40 mg/kg) | 0.306±0.01$^c$ | 0.212±0.01$^b$ | 0.28±0.01$^b$ | 0.32±0.01$^b$ |

Means ±S.E with different superscripts in the same column are significant at $P< 0.05$ using one way ANOVA test.

**Table (2):** Effect of oral administration of cypermethrin alone and/or in combination with vitamin E on sperm parameters of male rats.(Means ± S.E., n=5)

| Groups | Count ($10^6$/ml) | Motility (%) | Viability (%) | Abnormality (%) |
|---|---|---|---|---|
| Negative control | 75.0±1.7$^a$ | 91.6±1.9$^a$ | 90.2 ±1.5$^a$ | 7.6±1.2$^d$ |
| CYM (15 mg/kg ) | 44.0±0.9$^c$ | 56.0±1.9$^c$ | 53.0±1.2$^c$ | 21.4±0.6$^a$ |
| CYM (7.5 mg/kg ) | 47.0±0.1$^c$ | 58.0±2.1$^c$ | 50.0±1.5$^c$ | 17.6±0.2$^a$ |
| CYM (15 mg/kg ) + Vit. E (40 mg/kg) | 45.0±0.7$^c$ | 69.0±2.1$^b$ | 69.0±1.7$^b$ | 10.0±0.6$^b$ |
| CYM (7.5 mg/kg) + Vit. E (40 mg/kg) | 54.0±1.6$^b$ | 70.0±3.9$^b$ | 68.0±2.7$^b$ | 11.2±0.5$^b$ |

Means± S.E with different superscripts in the same column means significant difference at P< 0.05 using one way ANOVA test.

Activities in testicular tissue of male rats revealed that CYM induced significant decrease in the activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Concurrent administration of vitamin E and cypermethrin resulted in significant increases in the activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes as shown in Table (3).

**Table (3):** Effect of oral administration of cypermethrin alone and/or in combination with vitamin E on anti-oxidant enzyme activities in testicular tissue of male rats. (Means ± S.E., n=5)

| Groups | SOD (U/mg protein) | GPx (nmol/min/mg protein) | CAT (nmol/min /mg protein) |
|---|---|---|---|
| Negative control | 22.50±0.05$^a$ | 254.5±5.6$^a$ | 272.5±9.5$^a$ |
| CYM (15 mg/kg ) | 11.70±0.03$^d$ | 146.6±8.5$^e$ | 136.3±8.2$^e$ |
| CYM (7.5 mg/kg) | 14.50±0.02$^c$ | 158.6±7.4$^d$ | 160.2±7.5$^d$ |
| CYM (15 mg/kg ) + Vit. E (40 mg/kg) | 20.25±0.03$^a$ | 186.6±6.3$^c$ | 219.6±8.6$^b$ |
| CYM (7.5 mg/kg) + Vit. E (40 mg/kg) | 21.65±0.03$^a$ | 220.6±8.3$^b$ | 212.3±9.6$^b$ |

Means± S.E with different superscripts in the same column means significant difference at P< 0.01 using one way ANOVA test.

GPx unit = nmol of GSH utilized/min/mg protein.

CAT unit = nmol of H2O2 utilized/min/mg protein.

There were significant decreases in serum testosterone (TH), FSH and LH hormones levels in male rats when orally given cypermethrin at dose of 1/10 and 1/20 of $LD_{50}$ for 65 consecutive days. Concurrent administration of vitamin E with cypermethrin (CYM) when given to male rats at a dose of 1/10 of $LD_{50}$ for 65 consecutive days resulted in significant increases in serum levels of TH, FSH and LH.

**Table (4):** Effect of oral administration of cypermethrin (CYM) alone and/or in combination with vitamin E (Vit. E) on serum testosterone, FSH and LH in male rats. (Means ± S.E., n=5)

| Groups | Testosterone (mg/mL) | FSH (mg/mL) | LH (mg/mL) |
|---|---|---|---|
| Negative control | 3.90±0.04$^a$ | 2.20±0.037$^a$ | 0.38±0.012$^a$ |
| CYM (15 mg/kg ) | 2.7±0.01$^b$ | 1.62±0.06$^d$ | 0.313±0.001$^d$ |
| CYM (7.5 mg/kg) | 2.7±0.03$^b$ | 1.82±0.03$^b$ | 0.311±0.018$^d$ |
| CYM (15 mg/kg ) + Vit. E (40 mg/kg) | 3.6±0.01$^a$ | 1.72±0.05$^c$ | 0.323±0.004$^c$ |
| CYM (7.5 mg/kg ) + Vit. E (40 mg/kg) | 3.3±0.04$^a$ | 1.92±0.03$^b$ | 0.342±0.003$^b$ |

Means± S.E with different superscripts in the same column means significant difference at P< 0.05 using one way ANOVA test.

Histopathological examination of the testes showed degeneration of seminiferous tubules and edema in the interstitial tissue in the group given cypermethrin at 1/10 and at 1/20 of $LD_{50}$ as demonstrated in Fig. (1) and Fig. (2). the degenerated seminiferous tubules exhibited few layers of spermatogenic cells and few sperms in the lumen. The testes of rats given cypermethrin at 1/10 of $LD_{50}$ and vitamin E exhibited seminiferous tubules that were lined with spermatogenic cells up to sperm formation in the

lumen (Fig. 3). Rats administered cypermethirn at1/20 of $LD_{50}$ and vitamin E revealed a better sperm formation in the lumen of the seminiferous tubules (Fig. 4).

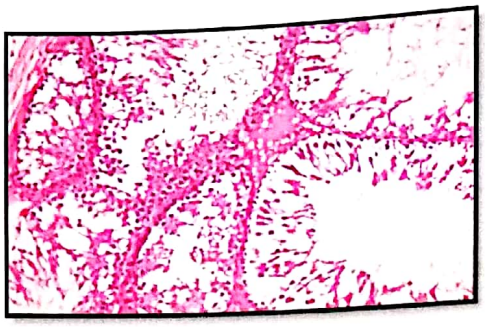

Fig.(1)

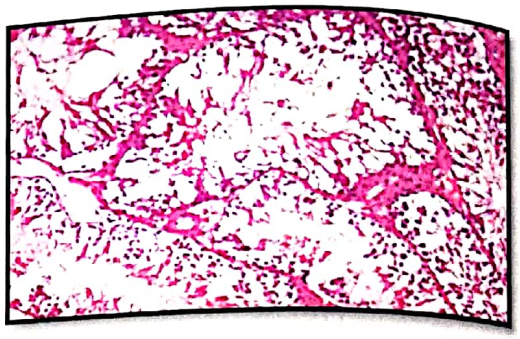

Fig.(2)

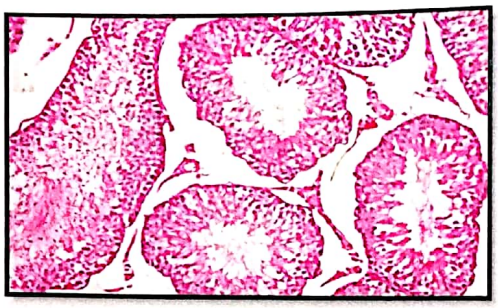

Fig. (3)

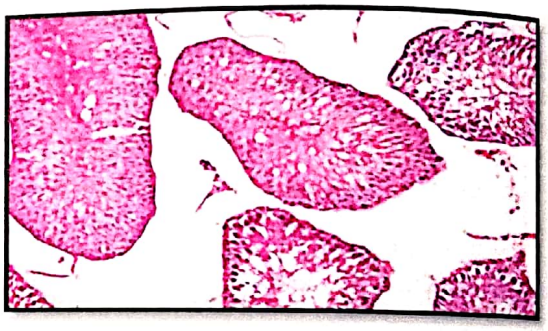

Fig. (4)

**Fig. (1): C.S.** of Testis of rat treated with cypermethrin 1/10 LD50 showing degeneration in the seminiferous tubules and mild degeneration in the interstitial tissue. (H & E X 200).
**Fig. (2): C.S.** of Testis of rat treated with cypermethrin 1/20 LD50 showing few layers of spermatogenic cells and few sperms in the seminiferous tubules. (H & E X 200).
**Fig. (3): C.S.** of Testis of rat treated with cypermethrin 1/10 LD50 and vitamin E showing seminiferous tubules lined by spermatogonial cells up to sperm formation. (H & E X 200).
**Fig. (4): C.S.** of Testis of rat treated with cypermethrin 1/20 LD50 and vitamin E showing seminiferous tubules lined by spermatogonial cells up to sperm formation. (H & E X 200)

## Discussion

In this study, oral administration of cypermethrin (CYM) alone at 15 and 7.5 mg/kg b.wt for 65 days to rats reduced male fertility, decreased genital organ weight, spermatozoa cell concentration, percentage of live spermatozoa and spermatozoa motility and increased percentage of total spermatozoa abnormalities with decreased plasma testosterone concentration. These findings were similar to those reported by Elbetieha *et al.*, (2001); Song et al., (2008); Wang *et al.*, (2009); Prakash *et al.*, (2010); Li and Cai, (2012) in rats and Al-Shaikh (2013); Al-Shaikh and EL Fayoumi (2013) in mice.

Concurrent administration of cypermethrin with vitamin E to male rats significantly evoked protective and antioxidant activities against toxic effect of cypermethrin. These protective effects were evident by significant increases in the relative weight of the testes, increases in serum levels of testosterone and FSH hormones, improvement of semen quality and quantity and alleviation the testicular degenerative lesions seen in the testes. The activity of antioxidant SOD and CAT enzymes was also increased in the testicular tissue. The protective and antioxidant activities of vitamin E against toxic effect of cypermethrin were in accordance with the previous findings (Jedlinska *et al.*, 2006).The previous authors concluded that vitamin E can protect sperm DNA from oxidative stress in the rat testis and enhance spermatogenesis and male fertility due

to its powerful antioxidant activity. The protective effect of vitamin E against cypermethrin toxicity on testicular histology and testicular enzymes in mice was also reported (Al-Shaikh, 2013 and Al-Shaikh and EL Fayoumi, 2013). Moreover, it has been found that combination of vitamin E and selenium improved semen parameters and pregnancy rates in infertile men as reported by (Moslemi and Tavanbakhsh, 2011).In the current study, the results concerning vitamin E were nearly similar to previous reports that vitamin E increased sperm count, motility and viability and increased serum testosterone, FSH, and LH levels. There were also alleviation of testicular degenerative changes including degeneration, congestion, and edema induced by lead acetate in vitamin E - administered rats.

The increase of serum sex (testosterone, LH and FSH) hormones levels caused by co-administration of vitamin E with cypermethrin, in this study, might be responsible for improving semen quality and quantity. It has been established that testosterone is essential for spermatogenesis, and also FSH plays a valuable role in germ cell progression and improves fertility in animal models (Sadettin and Tanzer, 2002).Moreover, it well known that the testicular function and spermatogenesis are controlled by FSH- and LH-linked mechanisms (Ballester et al., 2004).Administration of vitamin E with cypermethrin in combination induced antioxidant effect in the testes of rats. This effect was evident from the increased activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes. The reported antioxidant effect of vitamin E agreed with the previous findings (Jedlinska *et al*., 2006; Al-Shaikh, 2013 and EL Fayoumi, 2013).

serum sex hormone levels and decreased activity of testicular antioxidant enzymes. Coadministration of vitamin E with cypermethrin induces beneficial protective and antioxidant effects against reproductive toxicity induced by cypermethrin in male rats.

## Conclusion:

Oral administration of cypermethrin at doses of 15 and 7.5 mg/kg b.wt for 65 days to male rats causes reproductive toxicity. This toxicity is manifested by decreased weights of testes, lowered semen quality and quantity, decreased

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## الملخص العربي

**الدور الوقائي لفيتامين هـ ضد السمية التناسلية المحدثة بمبيد سايبرميثرين في ذكور الفئران**

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**شيماء رمضان امام - مروة صلاح خطاب وعزة محمد زكريا**

استهدفت هذه الدراسة تقييم تأثير مبيد الحشرات سايبرمثرين وذلك باعطائه عن طريق الفم منفردا أومتحدا مع فيتامين هـ على الأعضاء التناسلية الذكرية وعملية تكوين الحيوانات المنوية فى الفئران. وكذلك تم دراسة تأثير الدور الوقائى لفيتامين هـ بالإضافة إلى الفحص الهستوباثولوجى لأنسجة الخصية.وقد توزيع عدد ٢٥ فأر ذكر بالغ جنسيا إلى ٥ مجموعات احتوت كل منها على خمسة فئران. استخدمت الأولى كمجموعة ضابطة للتجربة وفى المجموعات الأربعة الأخرى تم اعطاء المبيد حشرى بجرعتين (١٥ و٧ مجم /كجم ) منفردا ومتلازما مع فيتامين هـ عن طريق الفم يوميا لمدة ٦٥ يوما. وتم اجراء التحليلات البيوكيميائية بالمصل وفحص خصائص السائل المنوى وإجراء الفحص الهستوباثولوجى للخصية. وأظهرت النتائج أن اعطاء فيتامين هـ مع سايبرمثرين عن طريق الفم لمدة ٦٥ يوم أدى الى تأثير واق ضد التأثيرات السامة للمبيد على الخصوبة وخصائص الحيوانات المنوية وادى الى زيادة معدل اكسدة الدهون الدهون. وفى الخلاصة فان اعطاء فيتامين ه مع المبيد الحشرى سايبرمثرين لمدة ٦٥ يوما له تأثيرات مفيدة على خصوبة ذكور الفئران وخصائص الحيوانات المنوية.