Toxic Effects of Administration of Cypermethrin, Vitamin E, Zinc and Their Mixtures on the Hormonal Levels of Thyroid, Kidney Functions and some Biochemical Parameters in Male Mice

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Abstract: The present study investigates the effects of cypermethrin (CYP) on thyroid gland function, serum lipogram, kidney function, some liver enzymes and brain acetylcholinestrase (AchE) activity in male mice. Cypermethrin was administrated orally to mice at 9 mg/kg b.wt/day for 4 weeks (Dose period) followed by 2 weeks of ceasing treatments (recovery period). Male mice were randomly divided into five groups of eight each: a control (untreated check group) (1), CYP-treated group (2), CYP (9 mg/kg/day) +vitamin E (18 mg/kg/day) (3), CYP + zinc (20 mg/kg/day) (4) and CYP+E+zinc (5). Results revealed that triiodothyronine (T3) and thyroxine (T4) were significantly decreased in CYP-group compared to control. In addition, the thyroid stimulating hormone (TSH) was also affected and recorded a significant increase by the treatments of CYP alone and CYP+vit E during the administration period. Also, significant increases of some biochemical parameters of kidney function (uric acid and creatinine) in serum were observed in CYP-group compared to control. The administration of zinc with CYP group (4) improved all parameters studied (uric acid, creatinine, T3, T4 and TSH) as compared with control. The level of total lipid was significantly decreased, while triglycerides, total cholesterol and HDL-cholesterol were significantly increased by cypermethrin administration. The activity of liver and serum gamma glutamyl transferase (GGT) were significantly increased after CYP administration, while the results showed marked reduction of liver glutathione (GSH) concentration and the activity of glutathione-s-transferase (GST). After cypermethrin administration with vitamin E, the activities of GGT, GST, and GSH level were significantly decreased compared to control. Moreover, inhibition percentage of brain acetylcholinestrase (AchE) activity was 62.98% by cypermethrin alone, but other groups (3), (4) and (5) had no effect on brain AchE activity. Results demonstrated the beneficial influence of vitamin E and zinc addition in combinations to reduce the harmful effects of cypermethrin.

Keywords: Cypermethrin, Vit E, Zinc, Thyroid hormones, Kidney functions, some biochemical parameters, male mice

INTRODUCTION

Pyrethroid pesticides are a group of man-made chemical products which are widely used in and around households as well as in agriculture. The use of pyrethroids has been increased during the past decade with the declining use of organophosphate pesticides, which are more acutely toxic to birds and mammals than pyrethroids (Bateman, 2000; Shafer *et al.,* 2004).

Cypermethrin is a synthetic pyrethroid which is highly used pesticide in agriculture, household and animal husbandry mainly to crack, crevice and spot treatment for insect control. Cypermethrin targets sodium channel along with magnesium and Apse in human body and animals. Cypermethrin is primarily mediated through hyper-excitation of the central nervous system. Additionally, cypermethrin induces neurotoxicity by modulating the level of gamma-amino butyric acid (GABA). Furthermore, cypermethrin mediated neurotoxicity is contributed by its ability to induce free radical generation (Aman *et al*., 2018). Cypermethrin has been extensively used in the developing countries, for combating agricultural pests and insects of veterinary as well as human concern (Assayed *et al.*, 2010a). The synthetic pyrethroid 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).

Zinc can be found in normal biological processes, including genetic expression, DNA synthesis, enzymatic catalysis, neurotransmission and apoptosis (Gumulee *et al*., 2011). Zinc is a dismutase superoxide cofactor, showing the chelating ability, thus stabilizing cell membranes, inhibiting lipid peroxidation and inducing metallothionein synthesis (Prasad, 2008). Zinc (Zn) as an essential trace element is necessary for animals. Zinc has been reported to have protective role in reducing the damage due to increase oxidative damage (Kang and Zhou, 2005). Zn is effective in stabilizing the cell membrane and prevents its oxidative destruction caused by free radicals. The antioxidant effect of Zn is mediated through the induction of metallothionein, which is a potent scavenger of toxic metals and hydroxyl radical (Kazi *et al*., 2012).

Vitamin E is an important antioxidant in biological systems (Nazirogln *et al*., 1999). Many authors studied the protective effects of vitamin E on the toxicant-induced organ damage in mammals (El-Sayed, 2001). Vitamin E in the mice plays an important role as a part of multicomponent antioxidant defense system. This system protects the cell against the adverse effects of reactive oxygen and other free radical initiators of the oxidation of polyunsaturated membrane phospholipids, critical proteins or both (Herman and Ferrans, 1983). Administration of vitamin E and selenium was reported to be useful in

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controlling cypermethrin induced oxidative stress in rats (Atessahin *et al*., 2005).

Some experimental studies have shown that vitamins C and E can be used to counteract pesticide toxicity (Yavuz *et al*., 2004; Yousef *et al*., 2006). Several biological defense mechanisms against intracellular oxidative stress are present in the organism such as antioxidant enzymes (glutathione reductase and glutathione-s-transferase) and non-enzymatic antioxidants such as vit E, vit C and glutathione which can also act to overcome the oxidative stress of the pesticides (Evans and Halliwell, 2001). Vitamin E (α- tocopherol) is a fat -soluble antioxidant in cells and protect cellular membranes and lipoproteins from peroxidation. In addition, several studies have indicated that vitamin E has an effective role in inhibiting the free radical formation and so reduce lipid peroxidation in biological systems (Uzun *et al.,* 2009). The previous study had shown that vitamin E deficiency can lead to infertility due to its polyphenol components rapidly generates free radicals and protect rat tissues (Jalili *et al.,* 2014). Vitamin E protects critical cellular structures against damage caused by oxygen-free radicals and reactive products of lipid peroxidation (Yousef, 2010).

Therefore, the present study was carried out to investigate the toxicity induced by cypermethrin alone and with vitamin E, with zinc and their combination by oral administration after 4 weeks. Thus, the present investigation aims to explore some physiological and biochemical changes in the levels of serum lipid profile, the thyroid hormones and antioxidant defense system under the influence of cypermethrin alone or mixed with vitamin E and zinc.

MATERIALS AND METHODS

Chemicals

-Cypermethrin (±) cynao-3- phenoxybenzyl-(±) cis, trans-3-(2,2-dichlorovinyl)-2,2,dimethyl cyclo-propane carboxylate (98%) [Philadelphia, PA, USA](CYP).

-Zinc chloride ($ZnCl_2$) powder (98% purity) was obtained from El-Gomhoria Co., Egypt (ZN).

-Vitamin E (DL-tocopheral acetate) used in this study on a protective agent was purchased from Pharco Pharmaceuticals, Alexandria, Egypt (Vit E). It was dissolved in corn oil with daily dose of 18 mg/kg mouse body weight as recommended by Saeed and El-Gendy (2000).

Animals

Eighty adult albino male mice (*Mus musculus*) (40-45 g) used in this experiment were obtained from the animal house of the Institute of Graduate Studies and Research, Alexandria Univ., Egypt. Animals were acclimatized to the laboratory condition for four weeks before being used. Animals were fed *ad libitum* with standard laboratory diet and allowed free access of water. All animals were maintained on a 12 hr light/12 hr dark cycle at constant temperature (25± 1°C).

Experimental design

The mice were divided randomly into five groups each of sixteen; eight of them were used for recovery period (2 weeks). The oral administration was daily done for 4 weeks and the followed protocol was as that seen in Table (1).

Table (1): Experimental mice groups and their protocol of treatments administration

Group No.	Groups administrated with	Dose
1	**250 µl corn oil /mice**	(Control)
2	**CYP**	9 mg/kg body weight/day
3	**CYP+ vit. E**	9 mg/kg/day + vitamin E (18 mg/kg/day)
4	**CYP + Zinc**	9 mg/kg/day + $ZnCl_2$ (20mg/kg/day)
5	**CYP+ vit. E+ Zinc**	9 mg CYP + 18 mg vitamin E + $ZnCl_2$ (20mg/kg/day)

Cypermethrin =CYP n=8

Mice of all groups were orally administered with suggested doses of the running treatments for four weeks (experimental period). The treatments were ceased after that for two weeks (recovery period).

Mice were then scarified at the end of experimental and recovery periods. Blood samples were collected in clean dry test tubes, serum were separated by centrifugation at 4000rpm for 20 minutes at 4°C and stored at -20°C pending analysis.

The determination of the evaluated parameters

Hormonal assay

Serum levels of thyroxine (T_4), Triiodothyronine (T_3) and Thyroid stimulating hormone (TSH) were determined by using enzyme-linked immunosorbent assays (ELISA) diagnostic kits obtained from Sigma Chemical Co., and have been done according to the method of Thakur *et al*. (1997) for thyroxine (T_4), Triiodothyronine (T_3), Morimoto and Inouye (1997) for Thyroid stimulating hormone (TSH).

Serum lipid profile

Serum total cholesterol and triglycerides were estimated by using diagnostic kit No.351 and 336, respectively obtained from Sigma Chemical Co., and have been done according to the methods of Allian *et al*. (1974), Fossati and Prencipe (1982), respectively. Levels of total lipids were determined according to the

method of Knight *et al*. (1972). Low density lipoprotein cholesterol (LDL-Cholestrol) and high density lipoprotein cholesterol (HDL-Cholestrol) were determined using Boehringer Mannheim kits (Germany) according to the methods of Freindewals *et al*. (1972), Burstein (1970), respectively.

Uric acid and creatinine measurements

Kits of uric acid and creatinine produced by Medical device safety services MDSS GmbH Burckhardts Tr 1 Hannover, Germany were obtained from Vitro Scient, Heliopolis, Cairo, Egypt, and the measurements were carried out according to the methods of Morgenstern *et al*. (1966), Mitchell (1973), respectively.

Determination of Glutathione-s-transferase (GST)

Each the control and treated livers of mice were weighted and homogenized in five volume (*i.e.* 5 ml/g wet tissue) of 0.1 M sodium phosphate buffer (pH 7.5) in a homogenizer. The homogenate was centrifuge for 60 min at 20000 rpm at 4ºC using Ultra centrifuge Glutathione-s-transferase (GST, EC 2.5.1.18) activity as described by Habig *et al*. (1974), using p-nitrobenzyl chloride as a substrate protein concentration and the enzyme was assayed following the method of Lowry *et al*. (1951). Gamma-glutamyl transferase (GGT) activity in liver was estimated according to the method of Young (1990).

Determination of Glutathione (GSH) concentration

The method used for the determination of (GSH) was essentially as same as that described by Richardson and Murphy (1975) with minor modifications. The liver tissue was homogenized in 10% perchloric acid and then centrifuged 3000rpm for 15 min in an Eppendorf centrifuge.GSH quantity was determined in the supernatant and protein quantity was determined in the precipitate. The GSH assay mixture contained 5.5-dithiobis-2-nitrobenzoic acid, DTNB (0.5 mM) dissolved in sodium phosphate buffer (pH 8, 0.1 M) and supernatant (10-20 µl).The mixture was incubated for 10 min at room temperature before the absorbance was read at the wave length of 412 nm. Protein in precipitate were redissolved in sodium hydroxide (1M) for 2h in a shaking water bath at 46ºC and their quantity was determined by the method of Lowry *et al*. (1951).

Determination of acetylcholinestrase (AchE)

The brains of control and treated mice were weighted and homogenized in ten volumes (*i.e.* 10 ml/g wet tissue) of cool 0.1 M phosphate buffer pH 8, using homogenizer for 50 seconds. The homogenate was then centrifuged at 8000 rpm for 20 min at 4ºC using Ultra-Centrifuge.

The supernatant was collected as the enzyme source for AchE and AchE activity was assayed by the method of Ellman *et al*. (1961) using acetylthiocholine iodide as a substrate. Protein content was assayed using bovine serum albumin as a standard.

Statistical analysis

Results are presented as mean ±standard error (S.E). Statistical analysis of the results was done according to the student –t-test (Hine and Wetherill, 1975) to find the differences between treated and control animals.

The percentage of change of each parameter from the corresponding control value was also calculated as the following formula:

% of change = [treatment – control / control×100

RESULTS AND DISCUSSION

Cypermethrin a pyrethroid pesticide is widely used in developing countries for pest control in agriculture, home and garden either alone or in combination with other pesticides (Jee *et al*., 2005; Sangha *et al*., 2013). The toxic effects of administration of cypermethrin, vitamin E, zinc and their mixtures on the hormonal levels of thyroid, kidney functions and some biochemical parameters in male mice were evaluated.

Effect of oral administration of cypermethrin alone, with vitamin E, with Zinc and their mixture on serum Triiodothyronine (T_3), tetraiodothyronine – thyroxin (T_4) and thyroid stimulating hormone (TSH) activity in male mice

Table (2) is showing the effects of cypermethrin alone (CYP), with vit. E (CYP+vit.E), with zinc (CYP+Zn) and their combination (CYP+ vit. E + zinc) on the activity of T_3, T_4 and TSH. Dosages were administrated daily for four weeks period and then the administration was ceased for two weeks as a recovery period. All the evaluated parameters were measured after the administration period and after the recovery period. Serum levels of T_3 and T_4 were significantly lower when cypermethrin alone was orally administrated (9 mg/kg.bwt/day) to mice showing change percentage of -31.91% and -47.45%, respectively as compared with control. On contrary, TSH was highly significant increased with the administration of cypermethrin alone (87.50%) and with vitamin E (91.66%), while group 4 (CYP+zinc) and group 5(CYP+vit. E+zinc) gave more or less the same values of TSH activity as that of control. These findings are in agreement with the data obtained by Girgis *et al*. (2000) who recorded an increase in TSH level accompanied by a decrease in T_3 and T_4 levels in rats.

The presented results are in accordance with those reported by Wang *et al*. (2002) and Finch *et al*. (2006) who found a decrease of both T_3 and T_4 and increase of TSH activity in the serum of the experimental rats exposed to different synthetic pyrethroid compounds.

Table (2): Effect of oral administration of cypermethrin alone, with vitamin E, with Zinc and their mixture on hormonal levels of thyroid gland (T_3, T_4 and TSH) in male mice after 4 weeks (dosage period) and recovery period (2 weeks after ceasing the administration)

Groups administrated with	Triiodothyronine (T_3) ng/dl			
	Dose period	(%) change	Recovery period	(%) change
Control	103.2±1.93	–	103.2±1.93	–
CYP	70.30±1.20*	-31.91	88.25±1.30	-14.52
CYP+ vit. E	90.25±1.27	-12.59	95.33±1.33	-07.67
CYP + Zinc	110.3±1.42	+06.82	99.25±1.27	-03.87
CYP+ vit. E+ Zinc	120.4±1.29	+16.61	100.3±1.20	-02.84
	Thyroxin (T_4)ug/dl			
Control	5.90± 0.30	–	5.90±0.03	–
CYP	3.10±0.45 *	-47.45	4.80±0.30	-18.64
CYP+ vit. E	4.80±0.50	-18.64	4.99±0.28	-15.42
CYP + Zinc	6.50±0.13	+10.16	5.10±0.33	-13.55
CYP+ vit. E+ Zinc	7.10±0.25	+20.33	5.30±0.35	-10.16
	TSH uU/ml			
Control	0.48±0.03	–	0.48±0.03	–
CYP	0.90±0.09**	+87.5	0.40±0.02	-16.66
CYP+ vit. E	0.92±0.08**	+91.66	0.46±0.01	-04.16
CYP + Zinc	0.50±0.09	+04.16	0.47±0.02	-02.00
CYP+ vit. E+ Zinc	0.49±0.05	+02.08	0.48±0.03	00.00

Each value is a mean ± SE where n=8.
Statistical difference from the control: * =significant at $p \leq 0.05$, ** =highly significant at $p \leq 0.001$.
% of change = [treatment – control / control×100.

Biochemical studies

Changes in some components of lipid profile

The present data in Table (3) indicated that serum total lipid was found to be decreased as a result of cypermethrin alone (9 mg/kg b.wt/day) administration after 4 weeks showing a change of -31.95% (less than control). On the other hand, the alterations induced in serum total lipid by cypermethrin with vit E, CYP with zinc and their combination appeared to be elevated giving higher percentages of +27.44%, +25.28% and +35.99% more than that of control animals, respectively. However, after the recovery period, the level of total lipid in all groups revealed a tendency to return to normal control values.

It has long been known thyroid hormones affect synthesis, mobilization and degradation of lipids with degradation being more influenced than synthesis as was reported by Pucci *et al*. (2000) and Asami *et al*. (2001). The hypolipidaemia was probably a result of mobilizing and degradation of lipids and increased accumulation of liver total lipids as was suggested by Abdel-Raheem *et al*. (1995).

The results in Table (3) indicated the effect of cypermethrin (9 mg/kg b.wt/day) on the serum triglycerides content where it was found to be increased (+82.05%) after 4 weeks of oral administration, following by an insignificant change by the treatment of cypermethrin with vit. E, but when zinc was supplemented with cypermethrin (group 4) there was no any significant effect compared to the corresponding control, showing the same results as that of control animals. Considering the effect of cypermethrin alone on the serum total cholesterol, it was found that their level significantly increased after 4 weeks of administration. Ghazouani *et al*. (2020) reported that α-cypermethrin (CYP) is a pyrethroid insecticide-like environmental pollutant, widely found in the environment. In addition, CYP was administrated at a dose of 8 mg/kg b.wt for 8 weeks by male Wistar rats. CYP caused a significant increase of 42% of that the concentration of the total cholesterol and more than 75% in triglycerides as compared to the control group.

Abdel-Razik (2018) evaluated the biochemical alteration associated with Imidacloprid (IMC) toxicity in male albino mice. The animals were orally administrated with (IMC) at a rate of 2.6 mg/kg b.wt/day for 28 days. The results showed that Imidacloprid administration caused a significant increase of total cholesterol, triglycerides and high density lipoprotein cholesterol (HDL-cholestrol).

The effect of tested treatments on kidney functions parameters

To study the effect of cypermethrin alone, with vit. E, with zinc supplementation and CYP + vit. E + zinc on kidney function, the concentration of uric acid and creatinine were determined after 4 weeks of administration (dosage period) and also they were determined after 2 weeks of recovery period.

Table (3): Effect of cypermethrin alone, with vitamin E, with zinc and their mixture on different types of lipid profile (total lipid, triglyceride, total cholesterol, HDL and LDL cholestrol) after 4 weeks (dosage period) and recovery period (2 weeks after ceasing the administration)

Groups administrated with	Total lipid mg/ dl			
	Dose period	(%) change	Recovery period	(%) change
Control	1028.1±4.2	–	1028.1±4.2	–
CYP	699.6±3.8*	-31.95	888.5±3.2	-13.57
CYP+ vit. E	1310.3±4.3	+27.44	920.8 ± 3.1	-10.43
CYP + Zinc	1288.1±3.9	+25.28	926.1± 3.4	-09.92
CYP+ vit. E+ Zinc	1398.2±4.3*	+35.99	998.7±3.1	-02.85
	Triglycerides mg/dl			
Control	105.3± 2.98	–	105.3±2.98	–
CYP	191.7±2.80**	+82.05	99.01±2.2	-05.98
CYP+ vit E	108.1±2.73	+02.65	100.7±2.8	-04.36
CYP + Zinc	105.3±2.13	00.00	106.5±2.5	+01.13
CYP+ vit. E+ Zinc	110.7±2.80	+05.12	103.3±2.7	-01.89
	Total cholesterol mg/dl			
Control	81.5 ± 1.2	—	81.5±1.2	—
CYP	113.3±1.8*	+39.01	91.3±1.1	+12.02
CYP+ vit. E	100.5±1.7	+23.31	80.5±1.3	-01.22
CYP + Zinc	90.3±1.3	+10.79	79.8±1.6	-02.08
CYP+ vit. E+ Zinc	73.5±1.8	-09.81	77.9±1.3	-04.41
	HDL – Cholesterol mg/ dl			
Control	23.6 ± 0.42	—	23.6±0.42	—
CYP	35.7±0.40**	+51.27	25.01±0.39	+05.97
CYP+ Vit. E	23.1±0.39	-02.11	23.5±0.41	-00.42
CYP + Zinc	24.8±0.41	+05.08	23.8±0.40	+00.84
CYP+ Vit. E+ Zinc	27.5±0.43	+16.52	23.5±0.42	-00.42
	LDL-Cholesterol mg/ dl			
Control	33.4 ± 1.25	–	33.4±1.25	—
CYP	38.7±1.01	+15.86	35.01±1.27	+04.82
CYP+ vit. E	33.8±1.17	+1.19	33.6±1.03	-00.59
CYP + Zinc	34.01±1.21	+1.82	33.9±1.21	+01.49
CYP+ vit. E+ Zinc	33.8±1.23	+1.19	33.4±1.21	00.00

Each Value is a mean ± SE where n =8.
Statistical difference from the control: * =significant at $p \leq 0.05$, ** =highly significant at $p \leq 0.001$.
% of change = [treatment – control / control×100.

Effects of cypermethrin alone, with vit. E, with zinc and their combination on uric acid concentration

The data presented in Table (4) show that cypermethrin alone caused significant change (an increase of +43.10%) in the concentration of uric acid, while the supplementation of zinc with cypermethrin caused little alteration in percentage of change. Also, Table (4) showed that the percentage of change after 4 weeks administration of cypermethrin + vit. E + zinc did not cause significant effects compared to the corresponding control.

Effect of cypermethrin alone, with vit. E, with zinc and their combination on creatinine level in the male mice serum

Table (4) demonstrated that the administration of cypermethrin alone after 4 weeks gave the highest creatinine level that reached 0.98 mg/dl, compared with the corresponding control value of 0.65mg/dl and the other running treatments (0.66-0.71 mg/dl).

CYP showed a highly significant value that was more than that of control by+50.76%. However at the same period after 4 weeks all groups 3, 4 and 5 did not cause any significant change in the level of creatinine. The current study show that cypermethrin alone increased creatinine and uric acid concentrations in the serum of treated male mice and these results agreed with those of Eissa and Zidan (2010). Elevation of creatinine and uric acid concentration in serum of treated male albino rats may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules (Walmsley and white, 1994). Creatinine and uric acid levels were used as biomarkers of kidney damages. Abdel-Razik (2018) evaluated the biochemical alteration associated with Imidacloprid (IMC) toxicity in male albino mice. Mice were orally administrated with) 2.6 mg IMC / kg b.wt for 28 days. The result showed that both urea and creatinine were increased. El-Maghraby and Taha (2012) found that exposing rats to Deltamethrin (DLM) induced significant increase in urea and creatinine. They showed that vitamin E can act as an effective antioxidant for DLM pesticide toxicity by reducing oxidative stress burden.

Table (4): Effect of cypermethrin alone, with vitamin E, with zinc and their mixture on kidney function through determination of uric acid and creatinine in serum of male mice after 4 weeks (Dosage period) and recovery period (2 weeks after ceasing the administration)

Groups administrated with	Uric acid mg/dl			
	Dose period	(%) change	Recovery period	(%) change
Control	5.80±0.48	–	5.80±0.48	–
CYP	8.30±0.46*	+43.10	6.20±0.39	+6.89
CYP+ vit. E	6.50±0.28	+12.06	6.30 ± 0.46	+8.62
CYP + Zinc	6.13±0.40	+5.68	6.09± 0.47	+5.00
CYP+ vit. E+ Zinc	5.83±0.46	+ 0.51	5.79±0.46	-0.17
	Creatinine mg/dl			
Control	0.65± 0.02	–	0.65±0.02	–
CYP	0.98±0.01**	+50.76	0.70±0.03	+ 7.69
CYP+ vit. E	0.70±0.03	+7.69	0.69±0.01	+ 6.15
CYP + Zinc	0.71±0.02	+ 9.23	0.67±0.02	+ 3.07
CYP+ vit. E+ Zinc	0.66±0.03	+ 1.53	0.65±0.01	0.00

Each Value is a mean ± SE where n=8.
Statistical difference from the control: * =significant at $p \leq 0.05$, ** =highly significant at $p \leq 0.001$.
% of change = [treatment – control / control×100.

Effects of cypermthrin, vit. E, zinc and their mixtures on liver and serum glutathione-S-transferase (GST), gamma glutamytransferase (GGT) and glutathione (GSH) level in male mice

Tables (5 and 6) show the effect of sub lethal dose of cypermethrin (9 mg/kg/day) alone, with vitamin E (18 mg/kg/day), with zinc (20 mg/kg/day) and their mixture (CYP+ vit E+ Zinc) as they were administrated for 4 weeks and ceased for 2 weeks (recovery period) on the detoxification system. The effect was assessed by determining glutathione (GSH) concentration, gamma-glutamyl transferase (GGT) and glutathione-S-transferase (GST) activities in animal's liver and serum. The results in Table (5) showed that both GST and GSH were significantly decreased (-36.80 and 20.92%, respectively) in liver tissue as the animal were administrated with CYP alone, while gamma glutamyl transferase (GGT) was significantly increased (+46.41%). The other running groups 3, 4, 5 were did not cause any significant effect in the GSH concentration and GST and GGT activity.

Table (5): Effect of oral administration of Cypermethrin alone, with vitamin E, with zinc and their mixture on glutathione -s- transferase (GST), gamma glutamyl transferase (GGT) and glutathione (GSH) level in male mice liver

Groups administrated with	GST umole/mg protein /h			
	Dose period	(%) change	Recovery period	(%) change
Control	9.51±0.43	–	09.60±0.40	–
CYP	6.01±0.39*	-36.80	10.30±0.31	+07.63
CYP+ vit. E	9.11±0.42	-04.20	09.47±0.41	-01.35
CYP + Zinc	9.01±0.38	-05.25	09.50±0.39	-01.04
CYP+ vit. E+ Zinc	9.39±0.40	-01.26	09.53±0.40	-00.72
	GGT u/g			
Control	13.53± 0.23	–	13.50±0.20	–
CYP	19.81±0.40*	+46.41	12.01±0.28	-11.03
CYP+ vit. E	13.08±0.21	-03.32	13.03±0.20	-03.48
CYP + Zinc	13.28±0.23	-01.84	13.20±0.23	-02.22
CYP+ vit. E+ Zinc	13.40±0.25	-00.96	13.44±0.25	-00.44
	GSH mg/g			
Control	181.85±0.81	–	180.81±0.51	–
CYP	143.79±0.53*	-20.92	173.23±0.49	-04.19
CYP+ vit. E	179.80±0.48	-01.12	175.05±0.53	-03.18
CYP + Zinc	180.30±0.51	-00.85	179.35±0.48	-0.80
CYP+ vit. E+ Zinc	180.50±0.53	-00.74	180.63±0.53	-0.09

Each Value is a mean ± SE where n=8.
* Statistical difference from the control: * =significant at $p \leq 0.05$,
** =highly significant at $p \leq 0.001$.
% of change = [treatment – control / control×100.

The present data are in agreement with those reported by El-Maghraby and Taha (2012) who found that the exposure of rats to Deltamethrin (DLM) induced significant decrease in glutathione-S-transferase (GST) enzyme activity and also GSH concentration. Das *et al*. (2016) evaluated the protective role of zinc in attending cypermethrin induced haematological toxicity and oxidative stress in erythrocytes of male rat. They recorded reductions of glutathione content and antioxidant enzymes of rat erythrocytes. Wang *et al*. (2016) stated that GST is belonging to a group of multigene and multifunctional detoxification enzymes and an important condition affecting GST expression is known to be oxidative stress.

Serum GSH level as well as the liver GSH content and GST activity were significantly lower than those of the control ones after cypermethrin treatment (Tables 5 and 6). On the other hand, the present data revealed that vitamin E administration causes improvement in the oxidative status induced by cypermethrin causing the increase of GSH level (+49.25%) and the activity of GGT and GST in serum were also increased +55.89% and +32.87%, respectively in Table (6).

These results are in agreement with those reported El-Maghraby *et al*. (2010) who showed that administration of the chlorpyrifos and deltamethrin (DLM) caused damage in rat liver and also caused decrease in the activities of GST and DOS enzymes. Reda (2005) found that the activity of gamma glutamyl transferase (GGT) was increased and a marked reduction in each of liver glutathione (GSH) level and the activity of glutathione-s-transferase (GST) were also recorded.

Ylmaz *et al*. (2003) reported that selenium may have an antioxidant effect in animal tissues against cypermethrin inducing oxidative stress, but vitamin E alone had no beneficial effect. Moreover, Gabbianelli *et al*. (2004) found that the treatment with vitamins E and C maintained the activity of glutathione-s-transferase (GST) in liver rat, while GST was significantly decreased due to chloride cadmium administration (EL-Demerdash *et al*., 2004).

Table (6): Effect of oral administration of Cypermethrin alone, with vitamin E, with zinc and their mixture on glutathione -s- transferase (GST), gamma glutamyl transferase (GGT) and glutathione (GSH) level in male mice serum

Groups	GST enzyme µmole/mg protein /h			
	Dose period	(%) change	Recovery period	(%) change
Control	24.85±1.25	–	24.45±1.05	–
CYP	31.19±1.11*	+25.51	23.95±1.02	-2.04
CYP+ vit. E	33.02±1.03*	+32.87	24.01±1.03	-1.79
CYP + Zinc	24.80±1.13	-0.11	24.13±1.05	-1.30
CYP+ Vit. E+ Zinc	24.05±1.20	-3.21	24.30±1.02	-0.61
	GGT u/g			
G Control	261.40±1.23	–	262.38±1.20	–
CYP	403.45±1.20**	+54.34	259.40±1.22	-1.13
CYP+ vit. E	407.50±1.31**	+55.89	260.39±1.18	-0.75
CYP + Zinc	270.13±1.29	+3.33	261.40±1.20	-0.37
CYP+ vit. E+ Zinc	260.49±1.30	-0.34	262.09±1.30	-0.11
	GSH mg/g			
Control	128.09±1.20	–	127.90±1.22	–
CYP	80.89±1.01*	-36.84	126.53±1.20	-1.07
CYP+ vit. E	191.18±1.12*	+49.25	125.95±1.22	-1.52
CYP + Zinc	128.01±1.20	-0.06	127.50±1.21	-0.31
CYP+ vit. E+ Zinc	128.07±1.23	-0.01	127.80±1.20	-0.07

Each Value is a mean ± SE where n=8.
Statistical difference from the control: * =significant at $p \leq 0.05$, ** =highly significant at $p \leq 0.001$.
% of change = [treatment – control / control×100.

The effect of evaluated treatments on Acetylcholinestrase (AchE) activity was investigated. Acetylcholinestrase (AchE) activity of control and other experimental groups are presented in Table (7). It is obvious from the data that cypermethrin treatment had an inhibitory effect on this enzyme extracted from brain tissue (-62.98%) but, other treatments groups (3, 4 and 5) did not cause any significant effects compared the corresponding values of control animals. After the recovery period (2 weeks post-ceasing treatments), the data of treatments showed that they had no effect on AchE.

These results are in agreement with those obtained by El-Maghraby and Taha (2012) who found that exposuring rats to deltamethrin (DLM) induced significant decrease in GST enzyme and meanwhile acetylcholinestrase (AchE) enzyme was inhibited. It has been observed that cypermethrin induced neurotoxicity through free radical formation, reducing the antioxidant defense mechanism, and inhibiting acetylcholinesterase (AchE) (Sharma *et al.,* 2014).

Table (7): Effect of Cypermethrin alone, with vitamin E, with Zinc and their mixture on brain Acetylcholinesterase (ACHE) activity in male mice

Groups administrated with	AchE activity v/g			
	Dose period	(%) change	Recovery period	(%) change
Control	62.17±0.79	--	61.90±0.80	--
CYP	23.01±0.80**	-62.98	60.80±0.55	-1.77
CYP+ vit. E	60.90±0.85	-2.04	60.51±0.70	-2.24
CYP + Zinc	61.01±0.60	-1.86	61.50±0.53	-0.64
CYP+ vit. E+ Zinc	62.02±0.65	-0.24	61.70±0.53	-0.32

Each Value is a mean ± SE where n=8.
Statistical difference from the control: * =significant at $p \leq 0.05$, ** =highly significant at $p \leq 0.001$.
% of change = [treatment – control / control×100.

Cypermethrin was found to display AchE inhibitory activity by interacting with the anionic substrate binding site. Sharma *et al*. (2014) found that the administration of resveratrol (a polyphenolic phytoalexin abundantly found in grapes and red wine) increased AChE activity and ameliorated cypermethrin-induced brain damage in Wistar rats.

Rehman *et al*. (2006) recorded an induction in the level of lipid profile and decrease in CAT activity following the administration of deltamethrin (DLM) to albino mice for a period of 15 days at doses of 5.6 mg/kg and 18mg/kg. Thus, significant increase in antioxidant enzyme level of experimental animals administrated with DLM+ vit. E was found as compared to those animals administrated with deltamethrin only; suggesting the protective potential effect of vitamin E.

The decrease in AchE activity could be due to the decrease of the enzyme synthesis by the inhibitory nature of a toxicant. Accumulation of pesticides in the liver is reported to disrupt lipid metabolism and increase serum cholesterol levels (Kalender *et al*. 2005). The inhibition of acetylcholinestrase (AchE) acitivity in the target tissues is the most important action of cypermethrin toxicity, AchE is an enzyme that catalyzes acetylcholine and prevents its accumulation at cholinergic synapses (Uzun *et al.,* 2009; Shah and Iqbal, 2010)

CONCLUSION

The present study was planned to evaluate sub chronic toxic effects of synthetic pyrethroids such as cypermethrin on kidney function, hormones of the thyroid, some bio-chemical parameters and antioxidant defense system. The current results demonstrated that male mice exposure to cypermethrin led to abnormal changes of certain biochemical parameters. Uric acid, creatinine and TSH were found to be significantly increased due to the treatment of cypermethrin alone. On the other hand, Triiodothyronine (T_3) and Thyroxin (T_4) were decreased. Lipid profile in serum male mice has been affected and ACHE was inhibited. Administration of zinc (20 mg/kg b.wt/ day) to male mice treated with cypermethrin exhibited a significant protective role against the oxidative stress. These data suggested that zinc supplementation is a good protective agent for the mammals that may subjected to cypermethrin intoxication and therefore, results demonstrated the beneficial influences of vitamin E in reducing the harmful effects of cypermethrin.

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التأثيرات السامة لتناول السيبرميثرين وڤيتامين E والزنك ومخاليطهم على نشاط هرمون الغدة الدرقية ووظائف الكُلى وبعض العمليات الكيموحيوية في ذكور الجرذان

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تعتبر البيريثرويدات المخلقة صناعياً من المبيدات واسعة الاستخدام في البيئة الزراعية ولذا أجريت هذه الدراسة بهدف معرفة التأثيرات السامة للسيبرمثرين على ذكور الجرذان البيضاء والتي اشتملت التأثير علي هرمونات الغدة الدرقية وأيضاً القياسات الكيموحيوية لوظائف الكلى و كذلك مستويات بعض الليبيدات وأيضاً إنزيمات الكبد وإنزيم الأستيل كولين استريز في المخ تحت تأثير التناول اليومي المستمر للسيبرميثرين (٩ ملليجرام/كجم من وزن الجسم/يوم) لمدة أربعة أسابيع متتالية يعقبها فترة تعافي لمدة أسبوعين آخرين يتم فيها إيقاف المعاملات. وتم تقسيم ذكور الفئران المأخوذة عشوائياً إلى خمس مجموعات كل مجموعة ثمانية فئران وهذه المجموعات هي مجموعة الكنترول غير المعاملة (م١)، المعاملة بالسيبرميثرين (٩ ملليجرام/كجم/يوم) (م٢)، المعاملة بالسيبرميثرين + ١٨ ملليجرام ڤيتامين E/كجم/يوم (م٣)، المعاملة بالسيبرميثرين + ٢٠ ملليجرام زنك /كجم /يوم (م٤) وأخيراً المجموعة المعاملة بالسيبرمثرين + ڤيتامين E+زنك (م٥)، وقد أوضحت النتائج ما يلي: **أولاً:** أدى تناول السيبرمثرين بمفرده إلي انخفاض معنوي في مستوى هرمونات الغدة الدرقية الثيروكسين (T4)، ثلاثي يود الثيرونين (T3) عند التقدير في نهاية فتره التناول وقد امتد هذا الانخفاض وأصبح غير معنوي بعد فترة التعافي مقارنة بالكنترول، وفيما يتعلق بالهرمون المحفز للغدة الدرقية فقد سُجلت زيادة ذات دلالة إحصائية بعد فتره التناول المزمن المستمر لمدة ٤ أسابيع في المجموعة المعاملة بالسيبرمثرين مضاف إليها ڤيتامين E وتبع تلك الزيادة حدوث نقص في مستويات الهرمون خلال فتره التعافي حتى تساوت مع قيم مجموعة الكنترول. **ثانياً:** أدي تناول السيبرميثرن بمفرده إلي حدوث نقص ملحوظ في مستوى الليبيدات الكلية وأيضاً ارتفاعاً معنوياً في الجليسريدات الثلاثية والكوليسترول الكلى والكوليسترول منخفض الكثافة، أما في حالة تناول السيبرمثرين المضاف إليه ڤيتامين E لم يحدث أي تغير معنوي في الجليسريدات الثلاثية وفى الكوليسترول عالي ومنخفض الكثافة. **ثالثاً:** في حاله تناول السيبرمثرين بمفرده حدثت زيادة معنوية في تركيز حمض اليوريك مع الكرياتنين ولم تحدث زيادة معنوية في جميع المجاميع المعاملة بالمخاليط التي كان الزنك أحد عناصرها. **رابعاً:** في حاله التناول المستمرللسيبرمثرين بمفرده حدث انخفاض في كل من إنزيم جلوتاثيون-س- ترانسفريز ومستوى الجلوتاثيون في الكبد مع حدوث زيادة معنوية في إنزيم جاما جلوتاميل ترانسفيريز. خامساً: أظهرت جميع المجاميع المعاملة مقارنة بالكنترول حدوث تثبيط غير معنوي لإنزيم الاستيل كولين استريز في حين أصبح التثبيط عالي ومعنوي في حاله المعاملة بالسيبرمثرين بمفرده بنسبة ٦٢,٩٨% ، مما سبق يتأكد الدور الوقائي لڤيتامين E والزنك في تقليل الآثار الضارة التي قد تنجم عن التعرض لمبيد السيبرمثرين.