Potential Impact of Zinc on Hazardous Effect of Pesticides in Male Rats

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Abstract: In the present study , fifty male rats (weighted 150 – 170 g) were divided into five groups(n=10) , the first group served as control, the second group was received acute dose of Azinphos methyl (4 mg/kg. b.wt./orally) and the fourth group was treated with Caprolin at dose of 100 mg / kg b. wt./orally. The third and fifth groups were treated orally with Zn So_4 (400 µ mol / kg b. wt.) daily for 6 weeks before pesticides treatment. Blood samples were collected at fixed time intervals of 72 hrs, 7 , 15 and 21 days after treatment with pesticides. The results of the current study showed deleterious damage due to Azinphos methyl and Caprolin administration ,represented in a significant decrease in serum zinc concentration , total proteins and albumin and significant increases in copper, iron , γ-glutamyl transferase , aspartate aminotransferase (AST) and alanin aminotransferase (ALT) levels, while the data revealed imbalance in thyroid function as a result of both pesticides administrations , which was showed in the enhancement of free T_4 level and significant decrease in free T_3 activity at 1 week (day 7) post-administration. Treatment of zinc sulphate (Zn So_4) pre-administration with Azinphos methyl or Caprolin attenuates to a great extent the damaging effects of two previous doses of pesticides on the assayed parameters except the thyroid hormones. Accordingly , Zinc treatment at the used dose may have indirect physiological effect on thyroid function.
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1. Introduction:

Free radicals can be generated either by metabolism of xenobiotics or by normal aerobic cellular metabolism (Slater, 1984). The peroxidation of lipids and proteins of cell membrane initiate the diverse effect of free radicals with the consequence of acute and chronic inflammatory reactions. These events are responsible for oxidative damage of the biological activity of different organs reflected by accumulation of abnormal products of oxidative intermediate compounds of metabolic pathways (Halliwell *et al.,* 1992).

The free radicals defense system, both enzymatic and non-enzymatic components, provides protection for the various cell compartments. The maintenance of cell integrity depends on the balance between the free radicals generation and the free radicals defense system. Imbalances may occur when increased free radicals generation overwhelms the defense system, or when the defense system is severely compromised and incapable of detoxifying the normal flux of increased production and accordingly, decreased detoxification will occur.

Pesticides induce oxidative stress leading to generation of free radicals and alterations in antioxidants or oxygen free radicals scavenging enzymes (Ahmed *et al.,* 2000). In this sense, various studies were carried out to investigate the effect of pesticides exposure on antioxidant enzymes and lipid peroxidation (Videla *et al.,* 2000). Additionally, Satoh *et al.* (1992) reported the toxicity of Paraquat mediated oxidative stress on lungs induce different pulmonary biochemical responses in rats. On the other hand, Lindane, Chlopyrifos and Paraquat induce reactive oxygen species result in series of changes that are reflected by enhanced lipid peroxidation, increased Lactate dehydrogenase leakage and decreased glutathion peroxide activity in different organs (brain, liver, lungs and kidneys). These reactive oxygen species may initiate oxidative damage leading to cytotoxic effects (Osman, 1999 and Ahmed *et al.,* 2000).

The ability of zinc to retard the oxidative processes has been recognized for many years. The mechanism of antioxidation of zinc can be divided into chronic and acute effects. The chronic effects involve exposure of an organism to zinc on along-term basis resulting in induction of some other substances that is the ultimate antioxidant such as the metallothioneins. Chronic zinc deprivation generally results in increased sensitivity to some oxidative stress (Bray and Bettger, 1990 and Garg *et al.,* 2007). The acute effects involve two mechanisms; protection of protein sulfhydryls (Gibbs *et al.,* 1989) or reduction of OH formation from H_2O_2 through the antagonism of redox active transition metals such as iron and copper (Fuller *et al.,* 1987). Thus, zinc may be capable of reducing cellular injury that might have a component of site specific oxidative damage such as post-ischemic tissue damage through a mechanism that might involve the antagonism of copper reactivity (Kappus *et al.,* 1985).

The objective of the present study is to evaluate the efficacy of zinc (as $ZnSO_4$) in hindering the toxic effects of two types of pesticides, Azinphos methyl (organophosphorus compound) and Caprolin (carbamate), which are used for treatment of rats to evaluate some biomarkers as indices for predicted oxidative stress. The current investigations deal with determination of trace elements such as zinc, copper and iron concentrations as well as the levels of γ-GT, AST, ALT, total proteins and albumin in serum. Also, free T3 and T4 concentrations were estimated in rats treated with either azinphos methyl or Caprolin solely or in combination with zinc.

2. Materials and Methods:

Experimental animals:

This study was carried out using fifty adult male albino rats weighing 150-170 grams obtained from the Animal House of Radioisotopes Application Department at Enshas, Egypt. The animals were kept under normal living condition and fed on standard diet.

Chemicals:

1- Zinc sulfate ($ZnSO_4$) was purchased from Sigma Chemical Co. and dissolved in saline solution with dilution according to the used dose.
2- Azinphoshos methyl (organophosphrus compound) pesticide with a chemical structure O , O - dimethyl S - [(4-oxo-l,2,3, benzotriazin -3 (4H)-γl methyl) phosphorodithioate] in powder form (98% purity) with defined LD_{50} equivalent to 16 mg/kg b. wt. dissolved in corn oil.
3- Caprolin (carbamate) pesticide with a chemical structure 1-aryl-naphthyl-methyl-carbamate in powder form (97% purity) with defined LD_{50} equivalent to 400 mg/kg b.wt. dissolved in corn oil.

Treatments:

$ZnSO_4$ were administered daily for 6 weeks orally by using stomach tube at a dose of 400 µmol/kg body weight (Satoh *et al.*, 1992).
Azinphos methyl was administered to rats with single .oral dose of 4 mg/kg b.wt ($^1/_4$ LD_{50}) whereas caprolin was administered orally with single dose of 100 mg/kg b.wt ($^1/_4$ LD_{50}).

Animals groups:

Rats were divided into equal five groups each of ten rats.

First group: Animals were administered saline as a vehicle and served as control group.

Second group: Animals were administered single dose of azinphos methyl (4mg/kg).

Third group: Animals were received ZnS04 (400 µmol/kg) daily for 6 weeks and a single dose of Azinphos methyl 1 hour after the last dose of $ZnSO_4$.

Fourth group: Animals were administered single dose of Caprolin (l00mg/kg).

Fifth group: Animals were received $ZnSO_4$ (400 µmol/kg) daily for 6 weeks and a single dose of Caprolin 1 hour after the last dose of $ZnSO_4$.

Samples collection:

Blood samples were withdrawn from orbital venous plexuses at 3,7,15 and 21 days from all groups after the last dose of treatment. The blood was centrifuged at 3000 rpm for 30 min to obtain serum and stored at -18°C for biochemical analysis.

Biochemical studies:

Serum zinc (Zn), copper (Cu) and iron (Fe) contents were measured by using atomic absorption spectrophotometer (210 VGP Buck, Scientic). Serum gamma glutamyl transferase (γ-GT), aspartate aminotransferase (AST) and alanin aminotransferase (ALT) activities, total proteins and albumin were assayed coloremetrically according to Reitman and Frankel (1957), Lowery *et al.* (1951) and Dumas and Biggs (1972), respectively. Serum free T3 was assayed according to Burger *et al.* (1982) and free T4 was determined according to Nicoloff *et al*. (1970) using radioimmunoassay technique.

Statistical analysis

All data were subjected to statistical analysis according to Snedecor and Cochran (1983). Treatment means were compared by Duncan test at 1% level of probability.

3. Results:

Data presented in tables (1) & (2) are concerning with the change patterns of zinc, copper and iron in both normal and treated rats\with Azinphos methyl or Caprolin and zinc sulphate in relation to time after administration.

The results revealed high significant increase in serum zinc level in rats administered single dose of Azinphos methyl on day 3 recording percentage amounting of +22.81 of the control level. This increase followed by gradual decrease from day 7 to day 21 reaching percentage decreases from control equal to -22.15 (Table 1). On the other hand, there was significant ($P<0.01$) difference of serum

zinc level in Caprolin treated group on days 15 and 21 with percentage decrease from control equal to -32.89 and -37.11, respectively (Table 2).

The treatment with of $ZnSO_4$ for 6 weeks with both pesticides modulates the serum zinc level to approximate the control value.

Table 1: Effect of zinc (400 µmol/kg b.wt.) and Azinphos methyl (4 mg/kg) on serum zinc, copper and iron levels.

Parameters	Days post-treatment	Control		Azinphos methyl		Zinc+azinphos methyl	
		Mean ± S.E	Change %	Mean ± S.E	Change %	Mean ± S.E	Change %
Zn (µg/dl)	3	175.31 ^{b} ±7.03	100	215.30 ^{a} ±3.01	+22.81	200.21 ^{a} ±4.11	+ 14.20
	7	174. 84 ^{bc} ±6.85	100	166.21 ^{a} ±6.18	-4.93	175.90 ^{b} ±5.30	+0.60
	15	176.01 ^{bc} ±7.89	100	140.17 ^{d} ±8.31	-20.63	176.26 ^{a} ±3.32	+0.14
	21	175. 88 ^{bc} ±7.03	100	135.15 ^{b} ±4.75	-22.15	!80.20 ^{a} ±4.51	+2.42
Cu (µg/dl)	3	60.51 ^{c} ± 2.3l	100	205.11 ^{a} ±2.34	+238.96	105.11 ^{b} ±2.0l	+73.70
	7	58.94 ^{d} ± 2.05	100	158.16 ^{c} ±3.81	+168.93	175. 17 ^{b} ±4.04	+197.20
	15	60.09 ^{c} ± 2.27	100	156.17 ^{b} ±3.93	+159.89	175.16 ^{c} ±5.8l	+191.49
	21	59.87 ^{c} ± 2.31	100	165.25 ^{b} ±2.03	+176.01	160.16 ^{b} ±4.73	+ 167.51
Fe (µg/dl)	3	805.21 ^{a} ± 6.58	100	973.96 ^{b} ± 9.03	+20.95	1000.11 ^{ab} ±8.81	+24.20
	7	816.71 ^{a} ± 6.67	100	1250.90 ^{b} ±7.17	+53.16	835.05 ^{a} ± 7.03	+ 2.24
	15	798.47 ^{a} ± 5.89	100	930.85 ^{a} ± 9.32	+16.57	895.03 ^{a} ± 5.20	+ 12.09
	21	819.06 ^{a} ± 6.40	100	895.81 ^{a} ±8.15	+ 9.37	870. 09 ^{a} ± 8.91	+ 6.23

Values have same superscript in the same raw are not significantly different (P< 0.01).

Table 2: Effect of zinc (400 µmol/kg) and Caprolin (100 mg/kg b.wt.) on serum zinc, copper and iron levels.

Parameters	Days post-treatment	Control		Caprolin		Zinc + caprolin	
		Mean ± S.E	Change %	Mean ± S'.E	Change %	Mean ± S.E	Change %
Zn (µg/dl)	3	175.31 ^{b} ± 7.03	100	165.18 ± 3.84	-5.77	185. 79 ^{b} ± 4.32	+5.05
	7	174.84 ^{bc} ± 6.81	100	165.71 ^{c} ± 4.23	-5.23	1 80.81 ^{bc} ± 5.31	+3.41
	15	176.01 ^{bc} ± 6.93	100	118.11 ^{c} ± 4.32	-32.89	185. 19 ^{bc} ± 6.01	+5.21
	21	175.88 ^{a} ± 7.22	100	110.61 ^{b} ± 3.02	-37.11	190.40 ^{a} ± 5.82	+8.25
Cu (µg/dl)	3	60.51 ^{c} ± 2.31	100	190.19 ^{a} ±3.02	+214.31	i90.19 ^{a} ± 4.33	+ 214.31
	7	58.94 ^{d} ± 2.05	100	l80.18 ^{ab} ±4.1I	+205.70	200.20 ^{a} ± 5.51	+ 239.66
	15	60.09 ^{c} ± 2.27	100	145.15 ^{b} ±3.85	+141.55	230.49 ^{a} ± 5.38	+ 283.57
	21	59.87 ^{c} ± 2.31	100	155.l6 ^{b} ± 4.15	+159.16	210.54 ^{a} ± 4.34	+ 25 1.66
Fe (µg/dl)	3	805.2l ^{a} ± 6.85	100	1860.09 ^{b} ±8.91	+131.00	1140.01 ^{bc} ±9.99	+ 41.57
	7	816.71 ^{a} ± 5.29	100	900.97 ^{b} ±7.43	+ 10.31	880.91 ^{ab} ± 9.67	+7.83
	15	798.47 ^{a} ± 5.99	100	820.94 ^{ab} ±6.97	+2.81	865.89 ^{ab} ± 6.90	+8.44
	21	8 1 9.06 ^{a} ±6.79	100	810.91 ^{ab} ±6. 33	+0.99	840. 86 ^{ab} ± 0.01	+2.66

Values have same superscript in the same raw are not significantly different (P<0.01).

On the contrary, administration of both pesticides increased significantly (P<0.01) the serum copper level when compared to the corresponding control. This change was more pronounced in rats administered Azinphos methyl than rats administered Caprolin. The maximum percentage increase from control was recorded on day 3 in both groups equal to +238.96 and +214.31, respectively.

The supplementation of $ZnSO_4$ before treatment increased the level of copper significantly (P<0.01) on days 3, 7, 15 and 21 and this effect was more pronounced in rats treated with Caprolin more than rats treated with Azinphos methyl.

Rats treated with Azinphos methyl in parallel with Caprolin treatment showed significant (P<0.01) increase compared to control in serum iron on days 3 and 7, which returned to normal level on days 15 and 21 post-treatment.

The administration of $ZnSO_4$ to rats can attenuate the increased iron level in groups treated with pesticides and maintain its normal level.

The results in tables (3 & 4) demonstrate the stronger harmful effects of Azinphos methyl than Caprolin on γ-GT enzyme. There is significant (P<0.01) elevation in γ-GT activity in serum of rats administered single dose of Azinphos methyl or Caprolin on days 3, 7, 14 and 21 recording percentage increase relative to control level of+83.04, +106.72, +72.48 and +122.88, respectively, in rats treated with Azinphos methyl and +48.84, +64.91, +52.36 and +41.64, respectively, in rats treated with Caprolin.

Oral administration of $ZnSO_4$ for 6 weeks to the rats before treatment with Azinphos methyl or Caprolin revealed significant modulation in γ-GT enzyme activity reflecting the beneficial action of zinc in reducing the harmful effects of both types of pesticides.

On the other hand, Azinphos methyl or Caprolin treatment increased the activities of transaminase enzymes (AST and ALT). During the experimental period, the percentage of AST activity in rats treated with Azinphos methyl was increased gradually from control level of +9.01% on day 3 to +21.68% on day 15 and returned to normal level on day 21 post-treatment. The same pattern of AST level was recorded in the rats treated with Caprolin, where the percentage changes were +4.93% , +13.72%, +13.07% on days 3, 7 and 15, respectively. With respect to the ALT concentration, there was significant (P<0.01) increase in all time intervals reached a maximum on day 7 post-treatment, where the percentage change was increased above the control by +59.23 % in rats treated with Azinphos methyl and +34.60 on day 15 in rats treated with Caprolin.

Administration of $ZnSO_4$ before treatments with both types of pesticides revealed significant (P<0.01) decreases in serum AST levels on days 7 and 15 and also in ALT values during all periods studied as compared with animals treated with pesticides only restoring values within normal levels.

Statistical analysis of data showed significant (P<0.01) inhibitory effect of both azinphos methyl and caprolin on serum total protein contents. The inhibitory effect was observed on days 3 and 7 post-treatment with azinphos methyl and on day 3 only post-treatment with caprolin. These effects followed by non-significant decreases at the end of experiment as compared with control group. This effect was more pronounced in animals treated with azinphos methyl than animals treated with caprolin. The percentage changes from control were -19.81% -20.39%, -11.91% and -1.86% on days 3, 7, 15 and 21, respectively, in rats treated with azinphos methyl. The percentage changes from control were -8.99%, -6.79%,-2.47% and-1% on days 3, 7, 15, 21, respectively, in rats treated with caprolin. The same pattern was noticed in albumin levels in groups treated with azinphos methyl oniy as shown in table (3), while non-significant changes were observed in albumin levels due to caprolin treatment (table 4).

The results showed that oral administration of $ZnSO_4$ to rats daily over 6 weeks before pesticides treatments improve the decrease in the total proteins and albumin contents when compared with control.

As summarized in tables (5) and (6), administration of azinphos methyl resulted in inhibition of free T3 level. This decrease was more pronounced on days 7 and 15 post-treatment and percentage changes were decreased from control by -17.72 % and -15.88 %, respectively. On the other hand, the level of free T4 showed significant (P<0.01) increase on days 3, 7 and 15 followed by non-significant increase which approaching the control level on day 21 post-treatment. However, the percentage change increases, relative to control, were +43.37%, +24.69% and +25.71% on days 3, 7 and 15, respectively. The data shows that administration of single dose of caprolin has non-significant effect on free T3 and T4 activities as compared to the corresponding control.

The synergistic effect of zinc and azinphos methyl was noticed. The activities of free T3 and T4 showed significant (P<0.01) increases which reached their maximum levels on days 15 and 7, respectively and approximated to their normal levels of control on day 21 post-treatment. The percentage change of T3 levels were increased from control by+43.55%, +37.34% and +68.23% and the percentage change of T4 levels were increased from control by +14.75%, +16.67% and +14.85% on days 3, 7 and 15, respectively.

The group administered zinc before Caprolin recorded significant (P<0.01) elevation in T_3 levels on days 3, 7 and 15 as compared to the group administered Caprolin only. The fluctuating pattern of free T4 level recorded percentage increases than control by +51.5%, +2.74%, +19.14% and +4.84% on days 3, 7, 15 and 21, respectively.

Table 3: Effect of zinc (400 µmol/kg) and azinphos methyl (4mg/kg) on serum liver enzymes, total proteins and albumin levels.

Parameters	Days post-reatment	Control		Azinphos methyl		Zinc + azinphos methyl	
		Mean ± S.E	Change %	Mean ± S.E	Change %	Mean ± S.E	Change %
Y-GT (U/L)	3	4.66^{b} ± 0.80	100	8.53^{a} ± 1.25	+83.04	5.40^{a} ±0.85	+15.80
	7	4.76^{a} ±0.75	100	9.84^{a} ±1.37	+ 106.72	4.91^{b} ±0.68	+3.15
	15	4.87^{C} ±0.83	100	8.40^{ab} ± 0.68	+732.48	5.42^{c} ± 1 .24	+112.9
	21	4.85^{b} ±0.81	100	10.81^{a} ± 1.97	+ 122.88	5.00^{b}±0.60	+3.09
AST (U/L)	3	33.41^{b} ±4.02	100	145.43^{ab} ± 5.33	+9.00	136.42^{b} ±4.49	+2.25
	7	$134.2l^{c}$ ±4.23	100	159.00^{a} ± 3.42	+ 18.47	44.49^{bc} ±3.56	+7.65
	15	132.65^{b} ±3.87	100	16 1. 42^{a} ± 4.08	+21.68	138.31^{b} ±4.39	+4.26
	21	131.89^{a} ±3.58	100	136.63^{a} ± 5.67	+23.59	128.25^{a} ±2.82	-2.75
ALT (U/L)	3	46.83^{b} ±1.46	100	59.72^{a} ± 3.87	+27.52	50.83^{b} ±3.13	+8.54
	7	45.60^{b} ±1.20	100	72.61^{a} ± 4.54	+59.23	$48.8l^{bc}$ ± 3.07	+7.o3
	15	44.88^{c} ±1.35	100	65.44^{a} ± 4.99	+45.81	48.46^{bc} ±2.09	+7.97
	21	45.06^{b} ±1.07	100	64.61^{a} ± 3.38	+43.38	47.45^{b} ±2.68	+5.30
Total Proteins (g/dl)	3	7.12^{a} ±1.25	100	5.71^{b} ± 0.33	-19.80	7.04^{a} ± 1.41	-1.12
	7	7.06^{a} ± 1.19	100	5. 62^{c} ± 0.32	-20.39	7.23^{a} ±2.09	+2.40
	15	6.88^{a} ±0.95	100	6.08^{a} ± 0.11	-11.91	7.1 8^{a} ± 1.45	+4.36
	21	6.97^{a} ± 1.06	100	6.84^{a} ± 0.39	-1.86	7.14^{a} ± 1.32	+2.43
Albumin (g/dl)	3	4.41^{a} ±0.95	100	3.44^{b} ± 0.25	-21.99	4.31^{a} ±1.22	-2.26
	7	4.37^{a} ±0.79	100	3.81^{b}± 0.37	-14.69	4.40^{a} ± 1.25	- 0.68
	15	4.50^{a} ± 0.77	100	3.90^{a} ± 0.25	-17.02	4.84^{a} ± 1.37	+7.55
	21	4.25^{a} ± 0.94	100	3.10^{a} ± 0.38	-37.09	4.44^{a} ± 1.45	+4.47

Values have same superscript in the same raw are not significantly different (P<0.01)

Table 4: Effect of zinc (400 µmol/kg) and caprolin (100 mg/kg) on serum liver enzymes, total proteins and albumin.

Parameters	Days post-treatmen	Control		Caprolin		Zinc + caprolin	
		Mean ± S.E	Change %	Mean ± S.E	Change	Mean ± S.E	Change
γ-GT (U/L)	3	4.66^{b} ± 0.80	100	6.43^{b} ± 1.51	+48.84	4.56^{b} ± 1.58	-2.14
	7	4.76^{b} ±0.75	100	7.85^{b} ± 1.37	+64.91	5.00^{b} ± 1.32	+5.04
	15	4.87^{c} ± 0.83	100	7.42^{b} ± 2.25	+52.36	6.00^{bc}± 1.52	+23.20
	21	4.85^{a} ±0.81	100	6.87^{b} ± 1.58	+41.64	5.66^{b} ± 1.25	+ 16.70
AST (U/L)	3	133.41^{b} ±4.02	100	140.00^{b} ±2.53	+4.93	I36.64^{b} ± 3.93	+2.42
	7	134.21^{a} ±4.23	100	152.63^{c} ± 4.42	+ 13.72	141.00^{bc} ± 4,61	+5.05
	15	132.65^{b} ±3.87	100	150,00^{a} ±3. 78	+ 13.07	!27.87^{b} ±5.71	-3.60
	21	131.89^{a} ±3.58	100	129.44^{a} ±2.27	-1.85	130.44^{a} ±5.81	-1.09
ALT (U/L)	3	46.83^{b} ± 1.46	100	54.00^{ab} ±3.38	+15.31	48.82^{b} ±2.15	+4.24
	7	45.60^{b} ± 1.20	100	59.98^{a} ±2.22	+ 31.53	48.42^{b} ±2.53	+6.18
	15	44.88^{c} ± 1.35	100	60.41^{a} ±3.36	+ 34.60	47.94^{c} ±2.87	+6.81
	21	45.06^{b} ± 1.07	100	58.76^{a} ±3.42	+ 30.40	50.12^{b} ±3.02	+ 11.22
Total Proteins (g/dl)	3	7.12^{a} ± 1.25	100	6.48^{b} ±1.33	-8.98	7.82^{a} ± 0.46	+ 9.83
	7	7.06^{a} ± 1.19	100	6.58^{a} ±1.26	-6.79	7.98^{a} ± 0.58	+13.03
	15	6.88^{a} ± 0.95	100	7.05^{a} ±1.35	-2.47	7.65^{a} ± 0.53	+ 11.19
	21	6.97^{a} ± 1.06	100	7.04^{a} ±1.57	-1.00	7.76^{a} ± 0.52	+ 11.33
Albumin (g/dl)	3	4.41^{a} ± 0.95	100	3.63^{a} ± 0.77	-17.68	4.69^{a} ± 0.77	+ 6.34
	7	4.37^{a} ± 0.79	100	3.88^{a} ± 0.74	-11.21	4.76^{a} ±0.66	+ 8.92
	15	4.50^{a} ± 0.77	100	3.98^{a} ± 0.89	-11.55	4.74^{a} ± 0.83	+ 5.33
	21	4.25^{a} ± 0.94	100	3.96^{a} ± 0.90	-6.82	4.76^{a} ±0.95	+12.00

Values have same superscript in the same raw are not significantly different (P<0.01).

Table 5: Effect of zinc (400 µmol/kg) and azinphos methyl (4 mg/kg) on serum free T_3 and T_4 levels.

Parameters	Days post-treatment	Control		Azinphos methyl		Zinc + azinphos methyl	
		Mean ±S.E	Change %	Mean ± S.E	Change %	Mean ± S.E	Change %
Free T_3 (pg/ml)	3	$1.63^{c} \pm 0.13$	100	$1.59^{b} \pm 0.08$	- 2.45	$2.34^{a} \pm 0.05$	+ 43.55
	7	$1.58^{d} \pm 0.12$	100	$1.30^{bc} \pm 0.03$	- 17.72	$2.17^{b} \pm 0.12$	+ 37.34
	15	$1.70^{b} + \pm 0.10$	500	$1.43^{b} \pm 0.04$	- 15.88	$2.86^{a} \pm 0.16$	+ 68.23
	21	$1.64^{c} \pm 0.11$	100	$1.60^{bc} \pm 0.11$	- 2.43 .	$1.72^{ab} \pm 0.18$	+ 4.87
Free T4 (ng/dl)	3	$3.32^{c} \pm 0.11$	100	$4.76^{b} \pm 0.17$	+ 43.37	$3.81^{abc} \pm 0.38$	+ 14.75
	7	$3.28^{b} \pm 0.10$	100	$4.09^{a} \pm 0.41$	+ 24.69	$3.83^{b} \pm 0.26$	+ 16.76
	15	$3.50^{c} \pm 0.10$	100	$4.40^{ab} \pm 0.26$	+ 25.71	$4.02^{b} \pm 0.32$	+ 14.85
	21	$3.30^{a} \pm 0.1\ 2$	100	$3.52^{a} \pm 0.38$	+ 6.66	$3.46^{a} \pm 0.10$	+ 4.84

Values have same superscript in the same raw are not significantly different (P<0.01).

Table 6: Effect of zinc (400 µmol/kg) and caprolin (100 ing/kg) on serum free T_3 and T_4 levels.

Parameters	Days post-treatment	Control		Caprolin		Zinc + caprolin	
		Mean ± S.E	Change %	Mean ± S.E	Change %	Mean ± S.E	
Free T_3 (pg/ml)	3	$1.63^{c} \pm 0.1\ 3$	100	$1.42^{bc} \pm 0.10$	-12.88	$2.36^{a} \pm 0.03$	+ 44.78
	7	$1.58^{d} \pm 0.12$	100	$1.50^{cd} \pm 0.04$	-5.06	$2.37^{a} \pm 0.06$	+ 50.00
	15	$1.70^{b} \pm 0.10$	100	$1.33^{b} \pm 0.37$	-21.76	$2.41^{a} \pm 0.18$	+ 41.76
	21	$1.64^{c} \pm 0.10$	100	$1.57^{bc} \pm 0.11$	-4.26	$1.87^{bc} \pm 0.11$	+ 14.02
Free T_4 (ng/dl)	3	$3.32^{c} \pm 0.11$	100	$3.82^{bc} \pm 0.35$	+ 15.06	$5.03^{a} + 0.19$	+ 51.50
	7	$3.28^{b} \pm 0.10$	100	$4.07^{ab} \pm 0.07$	+24.08	$3.37^{b} \pm 0.10$	+ 2.74
	15	$3.50^{c} \pm 0.10$	100	$4.11^{bc} \pm 0.19$	+ 17.42	$4.17^{a} \pm 0.21$	+ 19.14
	21	$3.30^{a} \pm 0.12$	100	$3.41^{a} \pm 0.17$	+3.33	$3.46^{a} \pm 0.07$	+ 4.84

Values have same superscript in the same raw are not significantly different (P < 0.01)

4. Discussion

Reactive oxygen species (ROS) may be involved in the toxicity of some pesticides (Videla *et al.,* 2000). These ROS may initiate oxidative damage leading to cytotoxic effects. Pesticides treatment induce disturbance in antioxidant enzymes functions leading to retardation of cellular activity and consequently, the function of organs will be affected (Osman, 1999). Since trace metals play a very important role in many biological activities, their imbalance may be contributed to organ dysfunction (Elnimr and Abdel-Rahim 1989).

According to the data of the present study, a moderate decrease in serum zinc concentration was recorded in treated rats accompanied by significant increase in copper and iron values, which was time dependent. These results confirmed the findings of Ferri *et al.* (2003). The decrease in zinc level may be attributed to release of zinc metal from damaged lymphoid organs and bone marrow after pesticides treatment and accumulation in the organs (Ashby and Tinwell *et al* , 1998). Also, pesticides administration to rats lead to oxidative stress characterized by glutathione (GSH) depletion (Videla *et al.,* 2000). Consequently, the induction of metallothioniene synthesis was enhanced (Nakagawa *et al.,* 1995) associated with zinc in different organs of the body (Powell, 2000).

The elevation in serum copper level observed in animals may be attributed to the marked reduction in ceruloplasium as a subsequent to the increase in activity of oxidative stress (Roxborough *et al.,* 2000). The copper complex protein failed to perform its function in binding with copper because 95% of copper is transformed by ceruloplasium.

It is well known that, when iron and copper are released from their carrier or storage proteins, the reduction was initiated resulting in high reactive hydroxyl radicals which enhance lipid peroxidation and hence tissue damage (Samokyszyn *et al.,* 1989). Further evidences support the concept that the magnitude of inflammation and oxidative stress play a crucial role in the regulation of the circulating trace elements and their carrier proteins (McMillan *et al.,* 2000). The excess iron present in serum may be due to the depressive action of pesticides on the

haematopoetic tissue, increase the erythrocytic destruction and direct destructive effect on red blood cell membrane itself which led to increase in the catabolism of haemoglobin (Zahran *et al.,* 2002).

Zinc sulphate supplementation to the treated rats partially normalized the serum levels of zinc and iron and raised the serum copper level. These data are in agreement with that of (Goel *et al.,* 2000 and Garg *et al.,* 2007) they studied the protective potential effect of zinc in modulating the toxicity of organophosphrus pesticides on trace elements concentrations in rats.

Both zinc and copper are co-factors in the metalloenzyme such as CuZn-superoxide dismutase, which provides the first line of defense against activated oxygen species by dismutation of superoxid anion radical (Bettger and O'Dell, 1981). The protective effect of zinc is known due to its inhibiting effect on the hydroxyl free radicals of metal ions (Cu and Fe) by competing for the binding sites of those ions (Powell, 2000) or through the induction of metalothioniene (Garg *et al.*, 2007).

The present study revealed significant elevation of γ-GT level due to azinphos methyl and Caprolin administrations and this increase was more pronounced in the group treated with Azinphos methyl.

More evidence is elicited the natural defense system represented by reduced glutathione, which is good index for the increased oxidative damage after oxidative stress induced by pesticides (Videla *et al.,* 2000, Ahmed *et al.,* 2000 and Lusini *et al.,* 2001). This concept was discussed on the basis that γ-GT has physiological role in counteracting the oxidative stress and the excessive production of free radicals by breaking down extracellular GSH and making its amino acids components available to the cells against the threat of glutathione depletion (Videla *et al.*, 2000). It was claimed that GSH depletion leads to induction of γ-GT. This argument gives evidence that the improved salvage of GSH can be obtained through increased activity of γ-GT, which has crucial role in the maintenance of intracellular GSH homeostasis and the regulation of cellular redox state (Rohman and Mac Nec, 2000).

In the present study, it was noticed moderate increase in serum AST level from day 3 to day 15 followed by decrease to reach its normal level on day 21 post-treatment. Meanwhile, serum ALT value continued in increasing trend till day 21 post-treatment. The present data support the several earlier reports that insecticides elevate the serum transaminases levels (Davalos *et al.,* 1996, Helal *et al.,* 1997, Goel *et al.,* 2000 and Heibashy and Amer, 2003). This elevation could be attributed to the disturbance in the metabolism of glutamic-glutamin system and/or destructive effect of pesticides on hepatic cells. It is worthy mention that ALT is more specific for hepatocellular damage than AST since AST is released from different organs such as heart, muscles and liver (Heibashy and Amer , 2003).

Another explanation was suggested by Shah and Gupto (2001) who attributed the increase in liver enzymes to pesticides exposure. The effects of pesticides on the cell membrane may be through the lysis of the lipid layer and/or intracellular organoids that leads to increased permeability and permit the passage of the enzymes into the blood.

Several reports pointed at the potential role of zinc in protecting the hepatic cells from oxidative stress induced by different agents and its effect in normalize to different degrees the activity of liver enzymes. The efficiency of zinc protection may be due to its antioxidant properties in the biochemical system through various specific mechanisms (Rashmi *et al.,* 2010).

Treatment with acute dose of azinphos methyl resulted in decrease in serum total proteins and albumin comparable to controls. Meanwhile, treatment with acute dose of caprolin didn't alter the proteins level but caused slightly decrease in albumin level on day 3 and 7 post-treatment. Oxidative stress of pesticides induced hepatotoxity and alteration in antioxidant enzymes (Videla *et al.,* 2000 and Ahmed *et al.,* 2000) with subsequent disturbance in the physiological role of the enzymatic system of the liver leading to inability of hepatic cells to synthesize albumin (Heibashy and Amer , 2003).

It is possible to suggest that the animals exposed to pesticides suffer from hepatic zinc deficiency associated with high iron accumulation in the tissues (Goal *et al.,* 2000). Iron accumulation can trigger tissue oxidative damage though its capacity to undergo redox cycling and participating in one-electron transfer reactions. These reactions promote the generation of OH from H_2O_4 (Fenton reaction) that oxidizes one or more amino acids (Stadtman, 1992) and the decrease of essential amino acids resulted in insufficiency of protein synthesis (Akenami *et al.,* 1997).

With respect to co-administration effects of azinphos methyl or caprolin with $ZnSO_4$, it is obvious that zinc treatment increase the level of total proteins and albumin to reach the control level or slightly more. These results are not surprising since the zinc has important role in the process of protein synthesis (Rashmi *et al.,* 2010). Zinc is required for the function of many intracellular proteins, including enzymes, transcription factors and proteins involved in DNA replication. Zinc has an effect on the epidermal growth factor that stimulate intracellular signaling that stimulate tyrosin phosphorylation of its

receptors (Tang and Shay, 2001). Moreover, zinc supplementation following depletion increase total proteins, total collagen and RNA/DNA (Parasad 1979).

From the present data, it is noticed that the activity of free T_3 was decreased and free T_4 was increased as a result of azinphos methyl and caprolin treatments. It is well known that the concentration of serum T_3 was lower than T_4 but T_3 hormone is more active, more loosely bound with proteins and degrade rapidly from the blood than T4 (Silvestri *et al.,* 2005). Otherwise, pesticides exert adverse effect on the morphological follicular cells or inhibit the action of enzymes responsible for the conversion of T_4 to T_3 (Ahmed *et al.,* 2000).

The increased hepatic metabolizing capacity is coupled with reduction in T_4 level. This decline is a result of pesticides exposure mediated extrathyroidally through chemical induction of hepatic thyroid hormone (Christenson *et al.,* 1995).

Also, it is clear from the obtained data that zinc has a synergistic effect with pesticides on thyroid gland which reflected by significant increase in serum free T3 level on days 3, 7 and 21 post-treatment. There is a suggested explanation depends on the indirect physiological correlation of zinc with synthesis of thyroid hormones. The pancreatic carboxypeptidases, which degraded the polypeptides into amino acids, are metalloenzymes that require zinc for its activity . The amino acids, mainly tyrosin, were incorporated into thyroid hormone through iodination process (Tang and shay, 2001).

The data obtained highlighted the general protection sustained by zinc against the oxidative damage induced by azinphos methyl and caprolin and also conducted to suggest that the manifestation of zinc protection involve distinct differences in thyroid hormones responses in rats treated with azinphos methyl or caprolin. Pervious studies supported that zinc has a potential role in mediating the toxic effects of pesticides. This important role of zinc may be due to its antioxidant property, ability to scavenge reactive oxygen species, ability to generate endogenous antioxidanls and its possible interaction with other trace elements in maintaining the cellular harmony.

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