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Effects of zinc supplementation on oxidant/antioxidant and lipids status of pesticides sprayers

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Abstract:

Background: Excess exposure to pesticides induces oxidative stress and causes alteration in the lipid profile
Objectives: The study aimed to evaluate the effects of Zinc (Zn) supplementation on the oxidant/antioxidant and lipid status in pesticide sprayers.
Methods: Forty pesticide sprayers were included in the study. Blood lipids, malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), and Zn were estimated; before and after Zn supplementation.
Results: Statistical analysis revealed that after Zn supplementation, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and MDA were significantly decreased. However, there was a significant increase in the high density lipoprotein (HDL), SOD, GPx, and Zn levels. After Zn supplementation, significant inverse correlations were detected between the Zn and the levels of MDA, TG, and VLDL, while positive correlation between Zn and the levels of HDL and TC.
Conclusions: Zn supplementation improves the oxidative/antioxidants and lipid status in pesticide sprayers.

Keywords: malondialdehyde, pesticide sprayers, serum lipids, superoxide dismutase, zinc supplementation

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Introduction

Pesticides have a significant public health benefit through decreasing food and vector-borne diseases. However, excess exposure to pesticides can induce oxidative stress by generating free radicals and altering the scavenging antioxidant enzymes [1, 2]. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzymes are the most important endogenous antioxidant enzymes. Malondialdehyde (MDA), a major oxidation product of peroxidized polyunsaturated fatty acids, has been considered as an important indicator of lipid peroxidation [3]. Oxidative stress is defined as disequilibrium between the peroxidants and antioxidants in the biological systems [4]. Lipid peroxidation has been used as a measure of oxidative stress induced by xenobiotics. It has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity [5]. While continuous exposure to pesticides is unavoidable, the use of different exogenous antioxidants could be effective in ameliorating the toxicity of pesticides [6]. Sometimes the endogenous antioxidant system becomes incompetent to scavenge the induced oxidative stress [7]. Zinc (Zn) is one of the most abundant trace elements in the body. It plays an important role in the structure and function of biological membranes and the antioxidant enzymes; such as SOD [8]. Zinc is required for enzymes involved in lipid synthesis and lipoprotein excretion. It is also known to have lipid lowering action [9]. Abdalla et al. [10] found that, the HDL was correlated with Zn levels but cholesterol was negatively correlated with Zn in pesticide sprayers. A previous experimental study demonstrated protective effects of some antioxidants; such as Zn, against the lipoperoxidative changes induced by pesticides in rats [11]. Highly reactive oxygen metabolites formed during pesticides' exposure act on the unsaturated fatty acids of phospholipid components of cell membrane to produce MDA. All these effects

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were inhibited by Zn supplementation [12]. Previous studies have reported that male rats that were chronically intoxicated with pesticides showed a decreased concentration of Zn in both liver and serum [13]. Also from previous literatures, GPx activity could be influenced by pesticide exposure [14]. Brocardo et al. [15] found that Zn supplementation is very important to restore GPx activity after pesticide exposure.

The protective mechanism of Zn against pesticide-induced dysfunction could be attributed to its important role in the regulation of cellular glutathione that is vital to cellular antioxidant defense [16]. This could be due to the greater utilization of glutathione for detoxification of electrophiles and free radicals produced from exposure to pesticides, and that Zn was proved to be inversely correlated with the levels of GPx and MDA in pesticide sprayers. Sahin et al. [17] proved that environmental stress caused by occupational pesticide exposure lowered serum concentrations of antioxidants; such as vitamins E, C and A, and minerals; such as Zn and chromium (Cr). Abdalla et al. [10] found that occupational exposure to pesticides could be responsible for hyperlipidemia and oxidative stress; especially among smokers, and they recommended mandatory antioxidant supplementation for the pesticide sprayers to improve their antioxidant status.

In Egypt, several types of organophosphorus pesticides were used; such as diazinon, malathion, chlorpyrifosmethyl, chlorpyrifos, and profenofos [18].

Malathion is a widely used organophosphate pesticide [19], and the most prevalent organophosphorus residue detected in the serum of Egyptian children. Moreover, organochlorines are still persistent in the environment, according to many studies in Egypt. High levels of DDT and hexachlorohexane have been detected in soil, water, milk, and fish samples at some Egyptian locations [20]. Abou-Arab [21] reported that the main organochloine residues in Egyptian aquatic ecosystem, vegetables and fruits were HCB, lindane, hepatochlor, DDT and its derivatives, aldrin and dieldrin. From previous study, lindane, o, p'-dichlorodiphenyldichloroethane (DDD) and total dichlorodiphenyltrichloroethane (DDT) were the only organochloine residues detected in significant high concentrations in the serum of Egyptian healthy females not occupationally exposed to pesticides [22].

The authors hypothesized that Zn decreases the toxic effects caused by pesticides. Therefore, the objective of the present study is to evaluate the effects of Zn supplementation on the oxidative/antioxidant status and the blood lipids among pesticide sprayers.

Subjects and methods

This study was conducted as a cross-section comparison study, comparing 80 pesticides sprayers from a small village located within an agricultural area in Upper Egypt (Fayoum) with the 80 control subjects not occupationally exposed to pesticides. The two groups were from the same socioeconomic status, and their ages were in the range of 20–66 years. From medical history and clinical examination, all the subjects with chronic diseases; such as diabetes, cancers, and cardiovascular diseases, were excluded from the two included groups from the beginning of the study.

Then a prospective clinical trial was conducted among 40 male pesticide sprayers, after exclusion of the pesticide sprayers with normal lipid profile. The pesticide sprayers were exposed to pesticides for more than 15 years (15–30 years), without wearing any personal protective equipment. They were using organophosphorus compounds regularly in two or three seasons per year. Each season lasted for 1 month.

The workers in the present study exposed to a mixture of organophosphorous pesticides as they involved in the spraying of different agricultural crops such as cotton, fruits, vegetables, etc., according to the agricultural season and the type of crop. They were also exposed to organochlorine residues in their foodstuffs as proved from the previous study [22].

Diet supplementation was done by providing film coated tablet contains 110 mg zinc sulfate from the Egyptian markets. The dose was one tablet daily for 1 month.

Written consents were taken from the included sprayers. Twelve hours Fasting blood samples were collected from each subject into two tubes, at the beginning of the study and after one month of Zn supplementation.

The collected blood samples were divided into two portions. The first part was left to clot and centrifuged to separate the serum, and the second portion of the blood sample was collected in heparin tubes for separation of packed RBCs. The serum was used for estimation of the blood lipids, the oxidative stress biomarker (MDA) and the Zn levels. The packed RBCs were used for estimation of GPx and SOD. The biochemical measurements were performed according to the details given in the kit's instructions.

Serum triglycerides (TG) were determined according to the method described by Fossati and Prencipe [23].

Serum total cholesterol (TC) was estimated according to the method described by Taylor et al. [24].

Serum low and high density lipoprotein viz., LDL and HDL were determined according to the method described by Wieland and Seidel [25]; Lopez–Virella et al. [26]. Also the serum very low density lipoprotein

(VLDL) was calculated from the equation formula of Warnick et al [27]. Procedure of Johnsen and Eliasson [28] was used in the determination of serum zinc (Zn).

Malondialdehyde (MDA) was estimated in the serum according to the method described by Satoh [29]. Glutathion peroxidase enzyme (GPx) was assayed by the method of Paglia and Valentina [30]. The activity of superoxide dismutase enzyme (SOD) in the erythrocyte lysate was estimated according to Haghighia and Weia [31].

Statistical analysis was done through SPSS version 18.0. The quantitative results were expressed as means ± standard deviation (SD). The Paired t-test was used to compare between the two dependent groups (before and after Zn supplementation). The correlation coefficient was used to study the relationships between quantitative variables. Percent change in the quantitative variables after supplementation were calculated compared to prior supplementation and illustrated in suitable figures. Level of significance was considered at p-value ≤ 0.05.

Results

There was no significance difference between the age of the pesticide sprayers and their controls (42.5 ± 13.1 and 43.5 ± 10.2 years, respectively). About 47.5% of the sprayers and 49.7% of the controls were smokers, without significant difference. All the examined sprayers worked for more than 15 years (15–30 years) with average 13.2 ± 4.1 years.

Before supplementation, the blood lipids of the sprayers were significantly higher compared to their controls, except HDL. HDL, SOD, GPx, and Zn of the sprayers were significantly lower compared to their controls (Table 1).

Table 1: Comparison of the blood lipids, the oxidative and antioxidant biomarkers between pesticide sprayers and their control groups.

Parameter	Controls (80)		Sprayers (80)		Independent t-test	
	Mean	SD	Mean	SD	t-test	p-value
TC, mg/dl	162.6	17.94	155.1	10.15	2.31	p<0.05
LDL, mg/dl	84.6	19.43	150.2	8.37	19.59	p<0.0001
TG, mg/dl	93.1	6.21	139.9	12.97	20.79	p<0.0001
HDL, mg/dl	49.8	5.23	44.0	8.61	3.642	p=0.001
VLDL, mg/dl	18.6	1.24	28.0	2.55	20.79	p<0.0001
MDA, nmol/ml	0.145	0.04	0.160	0.08	1.08	NS
SOD, U/g Hb	194.9	5.94	81.6	16.40	41.1	p<0.0001
GPx, U/g Hb	38.1	6.74	17.9	6.46	13.61	p<0.0001
Zinc, µg/dl	103.7	12.88	73.4	10.52	11.52	p<0.0001

[1] NS: non-significant

After Zn supplementation, serum levels of TC, LDL, TG, VLDL, and MDA were significantly decreased in the pesticide sprayers. While, HDL, SOD, GPx, and Zn levels were significantly increased (Table 2).

Table 2: Comparisons between markers of antioxidant, oxidative stress and blood lipid profiles in the sprayers before and after Zn supplementation.

	Before supplementation (40)		After supplementation (40)		Paired t-test	
	Mean	SD	Mean	SD	t-test	p-value
Antioxidants and MDA						
Zinc, µg/dl	73.4	10.52	88.6	8.44	17.48	p<0.0001
SOD, U/g Hb	81.6	16.36	92.5	7.26	5.03	p<0.0001
GPx, U/g Hb	17.9	6.46	24.2	8.82	9.5	p<0.0001
MDA, nmol/ml	0.16	0.08	0.09	0.05	5.38	p<0.0001
Blood lipids						
TC, mg/dl	162.6	17.94	138.8	9.31	22.26	p<0.0001

LDL, mg/dl	150.2	8.37	136	11.15	15.1	p<0.0001
TG, mg/dl	93.1	6.21	70.7	6.85	22.2	p<0.0001
HDL, mg/dl	44	8.61	62	9.15	14.43	p<0.0001
VLDL, mg/dl	18.6	1.24	14.1	1.37	22.2	p<0.0001

In Table 3 before Zn supplementation, SOD, GPx, and TC were positively correlated with Zn levels, while MDA was negatively correlated with Zn. Also, Zn, SOD, and HDL levels were significantly negatively correlated with MDA levels. On contrary TC, TG, and VLDL levels were significantly positively correlated with MDA levels. After Zn supplementation, negative significant correlations were detected between the Zn levels and the levels of MDA, TG, and VLDL. Also, there was a significant positive correlation between Zn and SOD, GPx, and HDL.

Table 3: The relationships between the levels of Zn and MDA and the antioxidants and the blood lipids in the sprayers (before and after Zn supplementation).

	Before Zn supplementation (40)				**After Zn supplementation (40)**	
	Zinc, µg/dL		**MDA, nmol/mL**		**Zinc, µg/dL**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**
Zinc, µg/dL	(a)		−0.4	p<0.05	(a)	
SOD, U/g Hb	0.5	p=0.05	−0.4	p=0.05	0.6	p<0.001
GPx, U/g Hb	0.4	p<0.05	−0.2	NS	0.5	p<0.005
MDA, nmol/mL	−0.4	p<0.05	(a)		−0.4	p<0.01
Blood lipids						
TC, mg/dL	0.5	p<0.001	0.4	p<0.05	0.5	p<0.001
LDL, mg/dL	−0.1	NS	0.3	NS	−0.3	NS
TG, mg/dL	−0.2	NS	0.5	p=0.05	−0.5	p<0.01
HDL, mg/dL	0.3	NS	−0.6	p<0.01	0.6	p=0.001
VLDL, mg/dL	−0.2	NS	0.4	p<0.05	−0.4	p<0.05

[1] NS: non-significant

According to the oxidative/antioxidant status, the highest improvement after supplementation was in MDA which was decreased by 41.1% of that before supplementation, followed by GPx that was increased by 34.8%, Zn and then SOD levels (Figure 1).

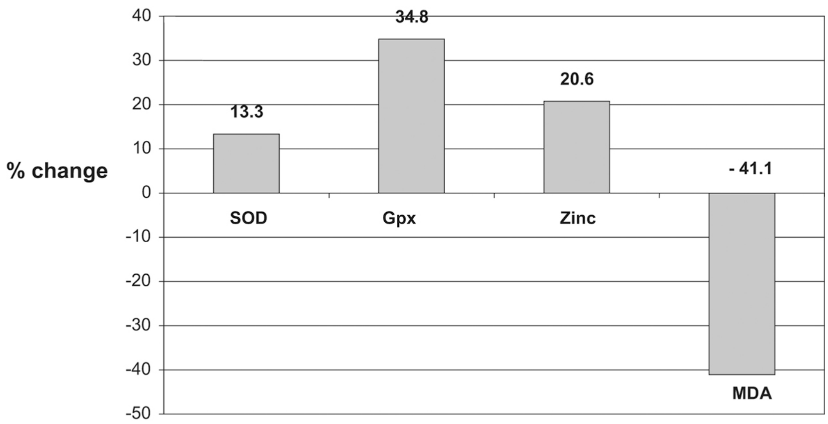

Figure 1: Percent change in the levels of antioxidant and oxidative stress biomarkers in sprayers after Zn supplementation.
SOD, Superoxide dismutase; Gpx, Glutathione peroxidase; MDA, Malondialdehyde.

According to the lipid profile, the highest improvement after supplementation was in HDL that was increased by 41% of that before supplementation, followed by TG and VLDL that were decreased by 24.1%. The improvements in other lipids were 10% or less (Figure 2).

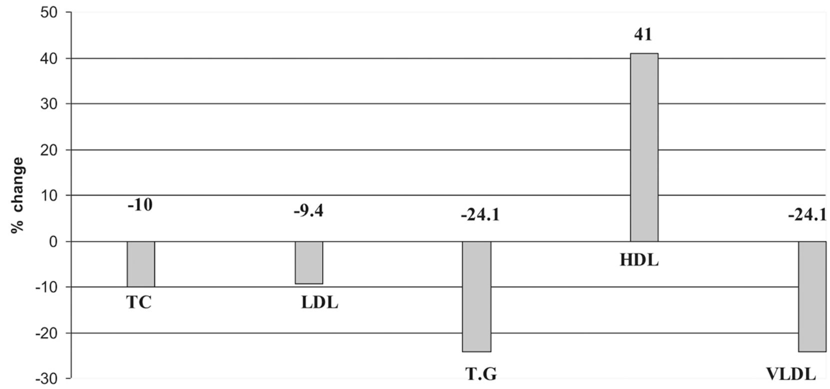

Figure 2: Percent changes in lipid profile of sprayers after Zinc supplementaion.
TC, Total cholesterol; LDL, Low density lipoprotein; TG, Triglycerides; HDL, High density lipoproteins; VLDL, Very low density lipoprotein.

Discussion

Several studies proved that pesticides induce oxidative stress that accelerate lipid peroxidation and antioxidant alteration [1, 32]. Pesticides may also increase tissue lipogenesis, that had been achieved through acceleration of acetyl CoA [33]. Increased lipogenesis could be the precursor of cholesterol biosynthesis. This may explain the significant correlations detected in the present study between the MDA and the levels of TC, TG, and VLDL in the pesticide sprayers before Zn supplementation. In addition, significant correlations between the Zn levels and the SOD, GPx, and TC, and significant inverse correlation between Zn and MDA were also detected.

Thus, the objective of this study was to evaluate the role of Zn supplementation on the oxidative/antioxidant status and the blood lipids in pesticide sprayers. Therefore, pesticide sprayers that had a significant elevated TC, TG, LDL, and VLDL, and significantly reduced levels of in the HDL, Zn, SOD, and GPx were included in the present study as compared to their controls.

It has been reported that the reactive metabolites of pesticides modify the activities of antioxidant enzymes in tissues after their exposure [34]. Also, they reported that it inhibit the SOD and GPx activity, and enhance the MDA production. The action of the antioxidants is directly neutralized by the formation of ROS and lipid peroxides. Some antioxidants reduce oxidative stress indirectly by addressing the sources of oxidative stress, and preventing the formation of ROS and lipid peroxides [35]. This reduction in oxidative stress, improves the lipid profile [36].

Zn supplementation protects against pesticides induced lipid peroxidation, and oxidative stress, as it is required as cofactor for a variety of antioxidant enzymes, particularly SOD [11]. In the present study after Zn supplementation, a significant increase was detected in Zn, SOD, and GPx levels, and significant decrease in the MDA in the pesticide sprayers. The significant improvement in the levels of Zn, SOD, GPx, and MDA were detected in different variations after Zn supplementations. The highest improvement was in the decrease in MDA levels and to a lesser extent in GPx, Zn, and SOD levels. This could be attributed to the exhaustion of Zn in the synthesis of the antioxidant enzymes SOD and GPx to be used for scavenging the excess of ROS and MDA. This was proved by the inverse correlation detected between the levels of Zn and the levels of MDA after supplementation. Thus, Zn supplementation is considered to be one of the best choices in case of exposure to the oxidative stress due to occupational or environmental exposure to pesticides.

In agreement with the current results, Abbassy et al. [12] revealed that pesticides caused a statistical significant decrease in SOD activity and elevation in MDA levels in rats, and Zn supplementation normalized the levels of SOD. They attributed their results to the importance of Zn as an essential component of SOD. Contradictory, Gradinariu et al. [37] detected a decrease in SOD after antioxidant supplementation rich in Zn (Zn 15 mg/capsule/day), and they attributed the lowered SOD activity in the protected groups to the increase in the capacity of the liver to neutralize herbicides toxic effects.

Zn is required for enzymes involved in lipid synthesis and lipoprotein excretion, and is also known to have lipid-lowering action [38]. Effect of alteration of serum zinc levels of plasma lipids is still controversial as low serum zinc was reported to be associated with low TC and TG but no change in LDL-C but Koo and Williams [39] reported elevated levels of TC, TG. The results of the present study revealed that, Zn supplementation for 1 month significantly decreases the levels of TC, LDL, TG, and VLDL in the examined pesticide sprayers, and significantly increases the HDL levels compared to before supplementation. These significant effects could be attributed to the direct and indirect actions of the Zn supplementation on the blood lipids and the oxidative/antioxidant status in pesticide sprayers. In agreement with the present results, Kadhim et al. [40] detected reduction in the levels of blood lipid after intake of Zn and antioxidants for 3 months. Also, significant negative correlations were found between serum Zn and TC, LDL, TG, and LDL/HDL cholesterol ratio; while a significant positive correlation was found between serum Zn and HDL cholesterol [41].

However, there was significant reduction in the TC in the pesticide sprayers after Zn supplementation in the present study, there was an unexpected significant correlation between Zn and TC in the sprayers before and after Zn supplementation. This significant correlation was also detected in previous studies. Taneja et al. [42] found the same significant correlation, and Hiller et al. [43] found that higher serum Zn levels were associated with higher levels of TC, LDL, and TG. But, contradictory Grzegorzewska and Mariak [44] found negative correlations between the Zn intake and the serum levels of TC.

Effect of alteration of Zn on TC, HDL, and LDL is still not clear with varying views [38]. Koo and Williams [39] proved that acute Zn depletion produced a significant reduction in total serum cholesterol; which was primarily due to the selective decline in HDL. This could explain the results in the present study considering the significant correlation between Zn and TC levels before and after Zn supplementation, as significant correlation was developed between Zn and HDL after Zn supplementation.

After Zn supplementation in the present study, inverse significant correlations were detected between the Zn levels and the levels of TG and VLDL. The blood lipids vary in response to the scavenging actions of SOD and GPx enzymes. HDL, TG, and VLDL were the most sensitive blood lipids, as the percent of reduction of these lipids after Zn supplementation showed the highest improvement compared to that before supplementation. This could be due to ROS-induced increase of the risk of hyperlipidemia, vascular, and glomerular damage to fall in the antioxidant capacity as mentioned by Moreno et al. [45]. The administration of antioxidants probably attenuates superoxide production or scavenges the already produced superoxide [46] and improves lipid profile.

Conclusion

Therefore, the use of exogenous Zn could be an effective mean to ameliorate the toxicity of pesticides, as Zn supplementation enhances the scavenging activities of antioxidant enzyme SOD and GPx against the oxidative stress in the examined pesticide sprayers, and consequently their blood lipids could be improved.

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