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# Research Article
# Protective Effect of Vitazinc on Chlorsan Induced Oxidative Stress, Genotoxicity and Histopathological Changes in Testicular Tissues of Male Rats

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## Abstract

**Background:** Chlorsan is highly effective bactericide and fungicide, so it is being widely used as insecticides in Egypt and the potential toxicity was raised more attention as it caused an oxidative stress and genotoxic effect in testicular tissue. Vitazinc, one of the most effective antioxidant and may play a role on preventing the toxic effect. **Materials and Methods:** Fourty mature Albino rats were divided into four groups (10 rats each). Group (1) control rats received orally an equivalent volume of corn oil on body weight. Group (2) rats in this group were orally administrated 1/10 $LD_{50}$ of chlorsan equal to 1 mg $kg^{-1}$ b.wt., dissolved in corn oil for 60 days. Group (3) male rat in the groups were orally administrated 1/10 $LD_{50}$ of chlorsan in dose level of 1 mg $kg^{-1}$ b.wt., beside 200 mg $kg^{-1}$ b.wt., vitazinc. Group (4) rats in the group received orally 200 mg $kg^{-1}$ b.wt., vitazinc only. **Results:** Chlorsan caused significant increase in lipid perioxidation. While significant inhibition in the activity of catalase (CAT), ChE activity and decrease in the level of reduced glutathione (GSH) were evident. Genotoxicity results revealed significant increase in the amount of m RNA of COX-2 and significant increase in the values of DNA fragmentation. Interestingly, pretreatment with Vitazinc attenuated these adverse effects. Vitazinc, therefore is a potent antioxidant and can protect against chlorsan-induced oxidative damage and genotoxicity by reducing lipid peroxidation and enhancing the antioxidant defense mechanisms. Histopathological examination revealed marked changes in testes of male treated rats. **Conclusion:** The present study reveals that vitazinc is effective in attenuating the oxidative stress, genotoxicity inflicted by chlorsan toxicit.

Key words: Vitamin E, zinc, pesticide, chlorsan, oxidative stress, ach, genotoxicity, reproductive toxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

In the recent years, significant consideration has been directed toward increasing exposure to environmental and occupational toxicants which might be connected with male-mediated developmental toxicity as sperm quality, implantation, the early embryo, low birth weight, congenital anomalies and neuro-developmental alterations[1].

Pesticides have been considered potential mutagens and some researchers have demonstrated that different agrochemical ingredients poss genotoxic properties leading to mutations, chromosomal alterations or DNA damage[2].

The pesticides featured in this study named chlorsan and belong to two different class of insecticides, organophosphate (chlorpyrifos, CPF) and pyrethroid (cypermethrin, CYP). Chlorpyrifos (o,o-diethyl-o-(3,5,b-trichloro-2-pyridyl) phosphorothioate), which is being widely used as insecticides in Egypt. It displays an expansive spectrum of activity against pests of plants, other animals including humans[3]. Like other organophosphate insecticides (OPIs), CPF poisoning occurs in primarily through the inhibition of acetylcholine-esterase activity in target tissues, resulting in accumulation of acetylcholine (Ach) at the cholinergic receptors in the peripheral and central nervous system[4]. Toxicity occurs at a dose which not inhibit AchE[5]. Therefore, other mechanisms have been implicated in OP toxicity such as induction of oxidative stress, which leading to generation of free radicals and a decrease in antioxidant enzymes or oxygen free radical scavenging systems[6]. Synthetic pyrethroids comprise a class of universal pesticides of which their usage increased. Cypermethrin (CYP), the alpha-cyano-3-phenoxy benzyl ester of 2,2-dimethyl -3-(2,2-dichlorovinyl)-cyclopropane carboxylic acid is the most widely used type II pyrethroid insecticides. Because of its low mammalian toxicity, CYP also used in controlling household pests. However, a several of investigations has suggested the hepatotoxic, carcinogenic, reproductive and immunosuppressive effects of CYP in mammals[7]. Currently oxidative stress and endocrine disruption are among the most vital subjects in pesticide toxicology[8].

The cell has several ways to mitigate the impacts of oxidative stress either by repairing the damage (damage nucleotides and lipid perioxide atoms by products) or directly by reducing the pro-oxidaive state via enzymatic and non-enzymatic antioxidants (vitamins and minerals), which have been shown to scavenge the free radicals and ROS[9]. Zinc is an antioxidant factor as it is a center constituent of free scavenging enzyme such as copper/zinc superoxide dismutase (SOD)[10]. Zinc plays an important role in transcription factor function and DNA repair[11]. Several investigations were carried on the protective effect of zinc against the toxicity of pesticides and metals in animals. Co-treatment with zinc partially improved the oxidative stress induced by deltamethrin in Albino rats and decreased the frequency of genetic mutation[12]. Additionally, some researchers stated that co-treatment with zinc normalized the deranged sperm parameters, hormonal profiles, minimized the evidence of testicular oxidative damage and reversed the impairment of spermatogenesis and steroidogenesis in Albino rats induced by the pesticides exposure[13]. The ameliorating effect of zinc against an acute hepatotoxic effect of chlorpyrifos in rats was conducted by researchers who revealed that co-administration and post treatment with zinc to chlorpyrifos (CPF) restored most biochemical parameters (liver function-oxidative stress) to with normal levels[14]. Vitamin E is the most critical lipid soluble antioxidant that protects the body from oxidative hazard. Many reports proved the reproductive protective role of vitamin E[15,16]. Oral administration of vitazinc (VE) attenuated the neurotoxic effect of deltamethrin (DM) through enhancement of oxidative status, DNA fragmentation percentage and suppressing the expression level[17,18] of CYP2EI, TP53 and COX-2. Thus, this study was aimed to explore (1) The possibility of oxidative stress and genotoxicity induction of chlorsan and (2) The possibility of protective effect of combination of vitamin E and zinc against the toxic effects of chlorsan on testicular tissue of adult male rats.

## MATERIALS AND METHODS

**Animals:** Sexually mature male Albino rats (weighing approximately 180-200 g) were housed in plastic cages, fed a standard laboratory diet and water. The animals were quarantined for 15 days before beginning the experiment. The animals were reared according to the principles of the "Guide for the care and Use of Laboratory Animals" prepared by Beni-Suef University. The Animal Care and Use Committee of Beni-Suef University approved the study. All efforts were made to minimize animal suffering.

**Reagents and chemicals:** Technical grade of chlorsan 29% which composed of 24% chlorpyrifos and 5% cypermethrin, was supplied by Kafer-EL-Zyat pesticides company, Egypt (KZ pesticides, company, Egypt). Vitazinc capsule was supplied by Egyptian INT pharmaceutical industries Co., each capsule contains, zinc gluconate 175 mg (Eq. to 25 mg zinc), vitamin A, 5000 IU and vitamin E 100 mg. Biodiagnostic kits for determination of catalase, activity of acetyl choline esterase,

glutathione and lipid perioxidation were obtained from Biodiagnostic Company Egypt.

**Experimental protocol:** Forty adult male Wister rats (weighing 180-200 g) were divided into four equal groups (10 rat each). Group (1) control rats received orally an equivalent volume of corn oil on body weight. Group (2) rats in this group were orally administrated 1/10 $LD_{50}$ of chlorsan equal to 1 mg $kg^{-1}$ b.wt., dissolved in corn oil for 60 days. Group (3) male rat in the groups were orally administrated 1/10 $LD_{50}$ of chlorsan in dose level of 1 mg $kg^{-1}$ b.wt., beside 200 mg $kg^{-1}$ b.wt., vitazinc. Group (4) rats in the group received orally 200 mg $kg^{-1}$ b.wt., vitazinc only. The oral $LD_{50}$ values of any pesticide are not equal and are dependant on the nature of pesticide along with the amount of pesticides exposed to the animals. Accordingly, the oral $LD_{50}$ of chlorsan, in particular for male rats is 10 mg $kg^{-1}$ b.wt.[19]. The reason for selecting a dose of 10 mg $kg^{-1}$ b.wt. (YIO $LD_{50}$) in the present study is due to its oral sublethal dose that caused toxicity to the animals and simultaneously did not cause mortality of the animals.

**Sampling:** At the end of the experiment, all animals were killed under light ether anaesthesia. Animals were rapidly dissected and blood was collected by cardiac puncture and serum was obtained by blood centrifugation at 1500×g for 10 min at 4°C.

**Biochemical analysis:** Testes were immediately removed, washed using chilled saline solution, then the testes tissue was perfused with phosphate buffer saline (50 mm potassium phosphate, pH 7.4, containing 0.16 mg $mL^{-1}$ heparin) to remove any red blood cells and clots. The tissue was homogenized in 5-10 mL cold buffer (i.e., 50 mM) potassium phosphate, which composed of 9.4 mL of 1 M monobasic solution and 40 mL of 1 M diabetic solution and complete with 1 L by distilled water, pH 5.1, 1 mM EDTA $g^{-1}$ tissue, using tissue homogenized and centrifuged at 4000 rpm/15 min at 4°C. The supernatant was washed and subjected to assay the activity of catalase[20], reduced glutathione concentration[21]. Lipid peroxidation (LPO) was estimated by measuring of thiobarbituric acid reactive substance (TBARS) according to method of Ohkawa *et al.*[22]. Acetylcholine esterase activity in serum was estimated by the method of Ellman *et al.*[23].

**Isolation of total RNA and real-time PCR (QPCR):** Total RNA was isolated from the testes by GF-1 total RNA extraction kit according to the manufactured instructions. The extracted total RNA stored at -80°C. Its yield and purity was assessed at 260 and 280 nm, respectively. The RT-PCR was performed by viva 2-steps. The RT-PCR kit according to the manufacture instructions for the COX-2 and GAPDH.

**Real time PCR:** The reaction mixture consisted of 1 Ul cDNA, 0.5 mm of each primer (COX-2 and GAPDH as internal control) which are illustrated in table, iQ SYBR green permix (BIO-RAD 170-880) in a total volume of 20 Ul. The PCR amplification and analysis were achieved using Bio-RAD I cycler thermal cycler and the MYiQ real time PCR detection system. The fast start polymerase was activated and cDNA denatured by a pre incubation for 10 min at 95°C, annealing of primers at 60°C programmed for 30 sec and extension at 72°C programmed for 30 sec fluorescent data were acquired during each extension phase[24]. The ΔCT value is calculated by the subtraction of the GAPDH CT from each COX-2 CT. The ΔCT value is calculated by subtraction of the control ΔCT from each COX-2 ΔCT. The expression relative to control is calculated using 2-ΔΔCT.

**DNA fragmentation assay:** The DNA fragmentation assay was conducted using DPA method according to Ojeda *et al.*[25].

**Histopathological study:** Specimens from the testes were taken and rapidly fixed in 10% neutral buffered formalin for at least 24 h the fixed specimen were processed through the conventional paraffin embedding techniques[26], sectioned at 5 μm and stained with hematoxylin and eosin (H and E).

**Statistical analysis:** Results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple test using SAS (SAS Institute Inc., Cary, NC). Data are presented as means plus or minus the standard error. The minimum level of significance was set at $p<0.05$.

## RESULTS

**Oxidative stress parameters:** The present data revealed that exposure of male rats to chlorsan induced significant inhibition in CAT activity and decrease in GSH level in the treated group compared to control one. Administration of vitazinc to chlorsan treated group improved the values of CAT and GSH toward the control values although the treatment could not normalize it. The greatest reduction was occurred in chlorsan-treated group. Treatment of male rats with vitazinc alone did not result in significant alteration in CAT and GSH values compared to the control group as shown in Table 1 and Fig. 1.

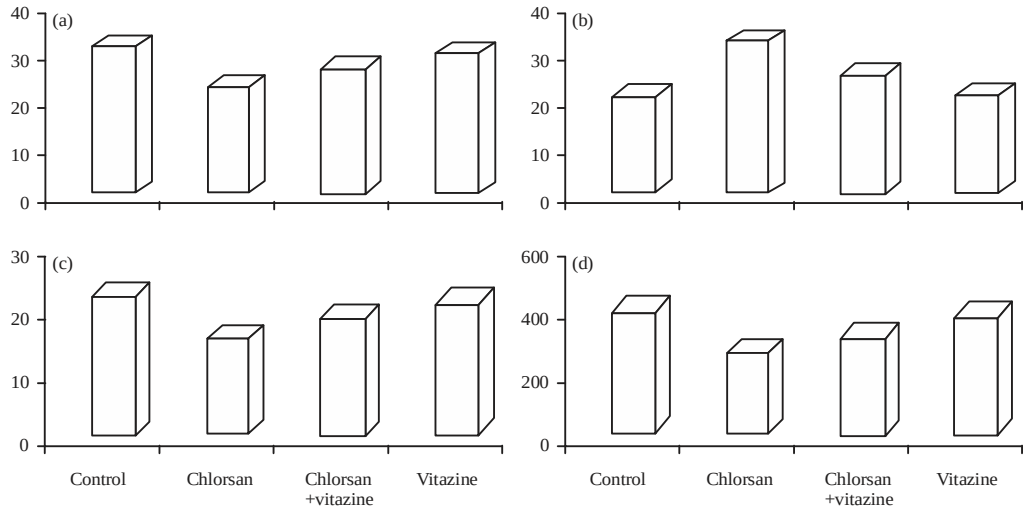


Fig. 1(a-d): Effect of chlorsan and its combination with vitazine on the antioxidant oxidative stress markers, (a) CAT (μ $g^{-1}$ tissue), (b) LPO (mmol $g^{-1}$ tissue), (c) GSH (mg $g^{-1}$ tissue) and (d) ChE (μ $L^{-1}$)

Table 1: Effect of chlorsan and its combination with vitazinc on the antioxidant and oxidative stress markers

| | Groups | | | |
|---|---|---|---|---|
| Parameters | Control | Chlorsan | Chlorsan+vitazinc | Vitazinc |
| CAT (μ $g^{-1}$ tissue) | 31.85±1.08 | 23.25±1.69** | 26.77±1.44* | 30.11±0.125 |
| GSH (mg $g^{-1}$ tissue) | 22.52±0.88 | 15.70±0.97** | 18.77±0.94* | 21.28±1.18 |
| LPO (mmol $g^{-1}$ tissue) | 21.01±0.20 | 32.59±0.66** | 25.33±1.04* | 21.18±0.98 |
| ChE (μ $L^{-1}$) | 402.66±4.78 | 266.12±20.65** | 316.42±9.05* | 386.62±14.07 |

Each value represent Mean±SE, *Significant differences versus control at $p \leq 0.05$, **Significant differences versus control at $p \leq 0.01$

Table 2: Primers used in real time PCR

| Gene | PCR primers | Size of PCR product (bp) | GenBank accession No. |
|---|---|---|---|
| Cyclooxygenase | F:5'AAGCCTCGTCCAGATGCTA-3' | 249 | NM_017232.3 |
| | R:5'ATGGTGGCTGTCTTGGTAGG3' | | |
| GAPDH | F:5'CGACCACTTTGTCAAGCTCA 3' | 153 | EU331417.1 |
| | R:5'CTGAGGGCCTCTCTCTTCCT 3' | | |

Table 3: Results of real time PCR

| Control group | Chlorsan group | Chlorsan+vitazinc group | Vitazinc group |
|---|---|---|---|
| 0.8 | 2.34 | 1.7 | 0.74 |

Data represent the Rq values

Level of lipid peroxidation (LPO) in testicular tissue, treatment of male rats with chlorsan resulted in a significant increase in the level of LPO (32.59±0.66) as compared to control value (22.52±0.88). Co-administration of vitazinc with chlorsan treated male rats induced lowering of LPO level but did not near that of control group. The greatest increase in LPO concentration was observed in chlorsan treated group. The serum activity of ChE was significantly inhibited ($p \leq 0.05$) in chlorsan treated group and in those co-treated with chlorsan+vitazinc ($p \leq 0.05$). The greatest inhibition in serum ChE activity was observed in chlorsan treated rats. Vitazinc trated rats had equivalent CAT, GSH, LPO and ChE values as that of the control group as shown in Table 1 and Fig. 1.

**Results of DNA fragmentation and RT-PCR:** Results of q-RT-PCR of COX-2 mRNA level of expression. The amount of mRNA of COX-2 showed 2.34 fold increase in the chlorsan treated group, whereas, the vitazinc group showed down expression. The vitazinc decrease the adverse effect of chlorsan in chlorsan vitazinc treated group by decreasing the level of expression into 1.7 fold as shown in Table 2 and 3.

The obtained data in this study revealed a significance increase in values of DNA fragmentation between control and chlorsan treated and vitazinc groups. While the group of chlorsan+vitazinc showed no significancy related to control one but there is significant decrease if compared to chlorsan treated group as shown in Table 4 and Fig. 2.

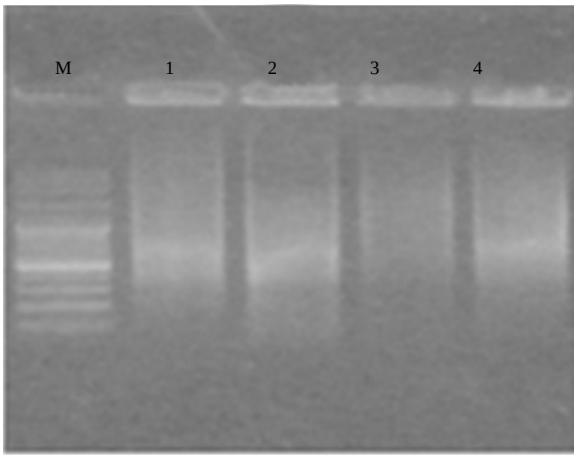


Fig. 2: Electrophoretic mobility of fragmented DNA on 2% agarosegel, Lane M: 100 bp DNA ladder, Lane 1: Control group, Lane 2: Chlorsan group, Lane 3: Vitazinc group and Lane 4: Chlorsan+vitazinc group

Table 4: DNA fragmentation percentages

| | Control group | Chlorsan group | Chlorsan+vitazinc group | Vitazinc group |
|---|---|---|---|---|
| DNA fragmentation (%) | 32.59±0.55 | 0.43±38.9** | 0.8±32.5 | 0.43±26.6** |

**Significant differences versus control at p≤0.01

**Histopathological findings in the testis:** Testicular section of control health rats and vitazinc treated rats had normal histoarchitecture that consist of uniform, well organized seminiferous tubules with complete spermatogenesis and normal interstitial connective tissue (Fig. 3a). Testicular tissue of rat received chlorsan alone showed degeneration and necrosis of the seminiferous tubules associated with the absence of the spermatogonial cells' series with appearance of few numbers of giant spermatogonia (Fig. 3b). The interstitial stromal connective tissue between the tubule showed focal hemorrhages (Fig. 3c). Conversely, rats received chlorsan plus vitazinc showed congestion in blood vessels between the tubules (Fig. 3d). There was a marked improvement of spermatogenesis, evidenced by the presence of elongated spermatids and spermatozoa in the majority of seminiferous tubules. Whereas, there was no histopathological alteration observed in vitazinc group (Fig. 3e).

## DISCUSSION

Organophosphate and pyrethroid are a group of pesticides that are commonly utilized in agriculture today. In the present study, we analyzed the protective role of vitazinc (vitamin E and A and zinc) against oxidative stress and genotoxicity, which prompted by chlorsan (chlorpyrifos+cypermethrin) in testicular tissue of male rats. The ROS is released during cellular respiration, processes of biodegradation, xenobiotic metabolism and phagocyte activation. However, the level of ROS may be significantly expanded by exposure to various environmental toxins including pesticides. It has been indicated that lipid peroxidation (LPO) is one of the molecular mechanisms involved in cytotoxicity of pesticide[27]. The present findings showed that the level of LPO was significantly increased in testicular tissues of rats exposed to chlorsan. Chlorsan induced increase in the level of lipid peroxidation is indicative of involvement of free radical mediated mechanism in its toxicity. The TBARS is a major oxidation product of peroxidized polyunsaturated fatty acids and increased TBARS level in the testicular tissue of treated male rats is in agreement with the findings of Attia *et al.*[28], Oda and El-Maddawy[29], Yousef[30] and Wang *et al.*[31] reported increase in LPO levels in testicular tissue of rats, rabbits and mice treated with the pesticide, chlorpyrifos, deltamethrin, lambda cyhalothrin and cypermethrin respectivel. El-Demerdash[32] demonstrated that the oxidative damage induced by pyrethroid may be due to their lipophilicity, whereby they could penetrate the cell membrane easily.

Organism posses the biological system to protect themselves against oxidative impacts caused by ROS production. For instance, to prevent oxidative damage, mammalian cells have built up a complex antioxidant system that induces non enzymatic antioxidant such as glutathione,

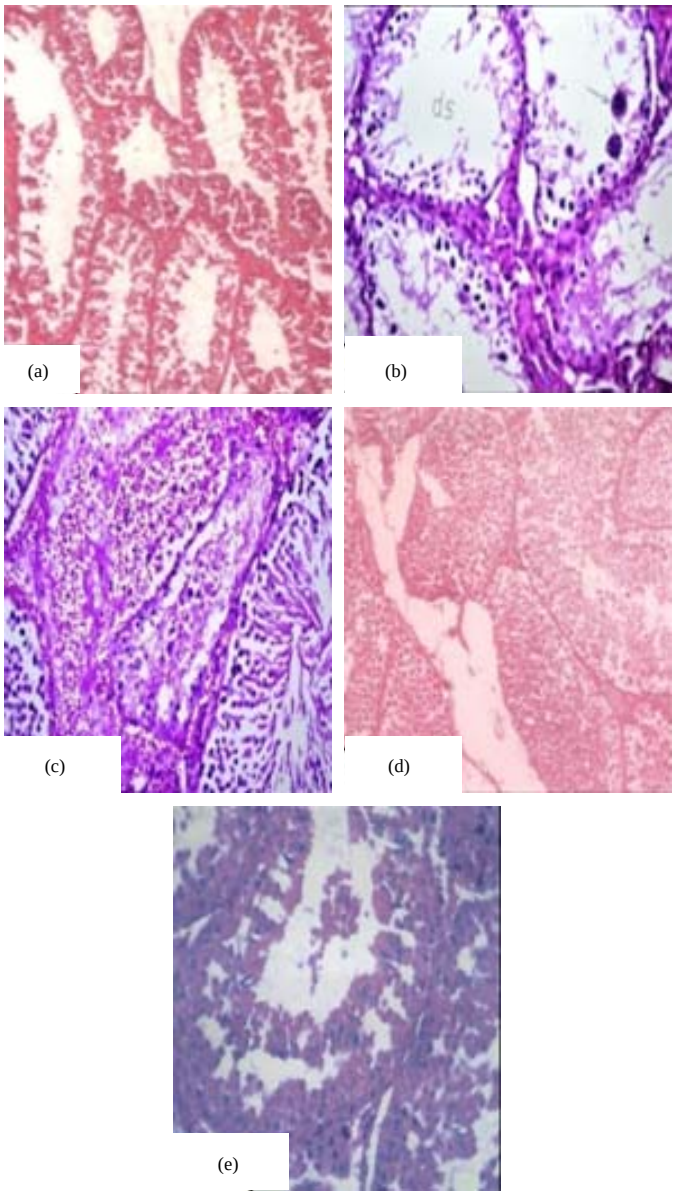


Fig. 3(a-e): (a) Testicular section of control health rats and vitazinc treated rats had normal histoarchitecture that consist of uniform, well organized semibeferous tubules with complete spermatogenesis and normal interstitial connective tissue, (b) Testicular tissue of rat received chlorsan alone showed degeneration and necrosis of the most of seminiferous tubules associated with absence of the spermatogonial cells series with appearance of few numbers of giant spermatogonia, (c) Interstitial stromal connective tissue between the tubule showed focal haemorrhages, (d) Conversely, rats received chlorsan plus vitazinc showed congestion in blood vessels between the tubules and (e) There was a marked improvement of spermatogenesis, evidenced by the presence of elongated spermatids and spermatozoa in the majority of seminiferous tubules. Whereas, there was no histopathological alteration observed in vitazinc group

ascorbic and alpha tocopherol and enzymatic antioxidant such as SOD, CAT and GPX[9]. The CAT converts hydrogen peroxide into water. These antioxidant enzymes, therefore, alleviate the toxic effects of ROS[33]. The data uncovered significant inhibition in CAT activity in testicular tissue of chlorsan male treated rats. These results are in parallel with Mansour and Mossa[33] who reported that CPF induced significant inhibition in CAT activity in testicular and liver

tissues of male rat and mice. Also El-Demerdash[34] found that CAT activity was significantly reduced in brain of rats exposed to the organophosphate fenitrothion. The GSH, considered as a second line of defense against xenobiotic substance. The GSH can scavenge a residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes[35]. A significant depletion of GSH was noted in the present study, this may be responsible for enhancement of LPO in testicular tissue of chlorsan treated rats. Several studies observed depletion of GSH content in different tissue of organophosphate and pyrethroid intoxicated animals[28,30,36,37]. It is well-known that testes contain very high level of glutathione than other organs, which plays an important role in the proliferation and differentiation of spermatogenic cells as concurrently protecting their cells from ROS damage[38].

The GSH in the testis acts either directly by scavenging the free radicals or acting as a substrate to GPX and GST during the detoxification of hydrogen peroxides and lipid peroxides as well as by preventing oxidative of SH-groups of protein[39]. The AchE activity, standard biomarker of organophosphate pesticide toxicity. The AchE activity was significantly reduced by 1/10 $LD_{50}$ of the tested insecticides chlorsan in testicular tissue of exposed rats. Okahashi *et al.*[40] reported that the inhibition in Ache activity occurs when animals intoxicated with different doses of fenitrothione for different periods. Actually the use of AchE inhibition as biomarker to assess the toxic effects of organophosphates has been studied for a wide range of species and many different xenobiotics and is a well-accepted index of organophosphate toxicity both *in vivo* and *in vitro*[41]. Pyrethroid have been demonstrated to cause a decrease in AchE erythrocyte and brain of living organisms[32,42]. The decrease AchE might be referred to the increase in lipid peroxidation as evident in the obtained data and the occupation of its active sites by pollutants. The decrease in AchE activity could lead to ionic refluxes and differential membrane permeability[43].

Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals. The consequences of the present study clearly demonstrated that chlorsan cause DNA damage in the treated rat testes as evidenced by a significant increase in DNA fragmentation and the amount of m RNA OF COX-2 expression. The OP compounds, methyl parathion, malathion, moncozeb, diazonin, chlorpyrifos and a cephate have been shown to cause DNA damage in different mammalian tissues and cells[44-46]. Chlorpyrifos has been appeared to be genotoxic to fresh water fish and caused DNA damage in different tissues[47]. The DNA fragmentation observed in the present study is the normal consequence of oxidative stress that was demonstrated as elevation in LPO, reduction in antioxidant enzyme (CAT) and glutathione content in the testes of rat. This is also consistent with previous studies where DNA fragmentation was induced by lambada-cyhalothrin in rat lymphocytes and liver[48,49] by cypermethrin in mice hepatocytes[50], rat lymphocytes[51], rat bone marrow[52] and rat brain[53,6]. Chlorsan being lipophilic in nature, so it can easily cross the cell membrane and may either interact directly with DNA or induce production of ROS capable of inflicting DNA damage as induced in the present study. Chlorpyrifos is a bifunctional alkylating agent because it has two ethoxy groups. Chlorpyrifos may alkylate both strands of DNA, leading to DNA interstrand cross links[50].

Alkylation of bases either directly or indirectly is a protein alkylation is probably involved in DNA damage[54]. The molecular mechanisms of the genotoxicity of cypermethrin are not yed elucidated and required further studies due to the hydrophobic nature and small molecular size, cypermethrin passes through the cell membrane and reaches the nucleus. It is proposed that within the nucleus cypermethrin binds to DNA through the reactive groups of its acid moiety, leading to destabilization as well as unwinding of the DNA, which could be possible mechanism for its genotoxicity[55]. Moreover, cypermethrin has both a vinyl and a dimethylcyclopropane group and the resultant active metabolite cause DNA damage[51]. Co-administration of vitazinc significantly enhanced the assessed parameters though not all were distinguished to control levels. Vitazinc ameliorated chlorsan induced oxidative damage and genotoxic effect on the testes of male treated rats. Vitamin E plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals who are toxic byproducts of many metabolic processes in biological membrane[55].

In this study co treatment of vitazinc with chlorsan induced significant reduction in LPO and as a consequence improvement in CAT activity and GSH level. Several studies demonstrate the protective mechanism of vitamin E against oxidative stress, in that alpha tocopherol reacts with peroxyl radicals depending on its methylation state of the chromosomal ring and the saturation grade of the side chain, forming tocopheroxyl radicals[56]. Vitamin E and A markedly decrease the DNA damage and the amount of mRNA of COX-2 in testicular tissue of chlorsan treated rats. In agreement with these findings, these reported by Aitken and Roman[10] who revealed that administration of mixed vitamin C, A and E with the pyrethroid markedly decreased the chromosomal aberrations. It was known that vitamins can prevent genetic changes by inhibiting DNA damage induced by reactive

oxygen metabolites[57,58]. Zinc is an essential trace mineral that acts as an antioxidant by neutralizing free radical generation[59]. It was suggested that Zn protection against the cytotoxicity of insecticides may be related to the maintenance of normal redox balance inside the cell[60]. Table 2 and 3 revealed that chlorsan treatment resulted in a significant increase in DNA fragmentation and COX-2 expression in testis of exposed animals, with a concomitant decrease in both parameters when compared to the control group.

Nevertheless, chlorsan and Zn treatment reduced these parameters when compared to chlorsan group only. However, significant decrease was observed in the DNA fragmentation and COX-2 expression level when compared to the control. The COX-2 which catalyzes the formation of prostaglandins from arachidonic acid is induced quickly by factors implicated in carcinogenesis, including growth factors, inflammatory stimuli oncogenes and tumor promoters. Some authors concluded that zinc supplementation might have important implications in cancer prevention, predominantly through suppression of NF-Kb signaling[61]. Furthermore, we noted that zinc supplementation was effective in reducing the over expression of the biomarkers COX-2 gene. The findings support the quest of zinc as a potential factor for genotoxic prevention and the up regulation of the biomarkers COX-2. A transcription complex known to regulate COX-2 gene expression via binding to CRE-sites in the promoter region is Activator Protein 1 (AP-1), which consists of homo or heterodimers of JUN and FOS families with roles in several different cancers. Primary goals of this study were to confirm chlorsan-sensitive transcriptional changes in COX-2 gene using qRT-PCR. Data from qRTPCR experiments demonstrated opposite the qualitative transcriptional response produced by chlorsan, which increases the COX-2 expression and Zn, which decrease the expression level in the testes.

The qRT-PCR data from chlorsan and zinc treatment studies were analyzed according to the 2-ΔΔCT method described by Livak and Schmittgen[24]. The present study has identified that the COX-2 transcription is altered that DNA fragmentation percentage is increased by insecticide in testes, suggesting the adverse action of these compounds on the reproductive organs. Zinc accumulates in the testis at high levels, which are comparable to those in liver and kidney. The inhibition of spermatogenesis and sperm abnormalities had been induced by Zn deficiency. *In vivo*, experiments in rodents have also demonstrated that a Zn deficiency can cause severe damage to the testes such as atrophy of the testicular tubules and the inhibition of spermatid differentiation. Moreover, there are some reports that exposure to Zn can alleviate testis damage by stress such as heavy metals, fluoride and heat due to that Zn may exert a protective effect against testicular injury and play an essential role in the maintenance of testicular function. High concentration of Zinc found in the testes and accessory sex glands show its pivotal role in the reproductive system. Zinc is essential in spermatogenesis, a cofactor of metalloenzymes, involved in DNA transcription, steroid receptor expression and protein synthesis.

Microscopic examination of paraffin section stained with H and E failed to reveal consistent differences in the histology of the testes of vitazinc alone treated animals when compared with that of the section of control animals. The histological changes in the testes of rats treated with chlorsan showed a marked histological alteration including severe degeneration and necrosis in most of the seminiferous tubules, associated with the absence of the spermatogonia cell series with the appearance of few numbers of giant spermatogonia. In parallel with these lesions those reported by Oda and El-Maddawy[29] and Al-Shaikh[62] On the contrary, the administration of vitazinc significantly reverted back the altered structure to near normal this might be due to free radical scavenging activity and potent antioxidant property of vitamin A and E and zinc.

These results were in parallel with data of Dirican and Kalender[63], they reported that vitamin E reduced the toxicity of dichlorofos on the composition of the testis tissue of male rats. Also vitamin E improved the testicular tissue as evidence by the presence of normal and natural leydig cells and sertoli cells[64]. In agreement with the results those reported by Oyewopo *et al*.[13] they mentioned that co-treatment with zinc prevented the cytotoxicity of the testes exposed to insecticide.

## CONCLUSION

The data of this experiment suggest that vitazinc positively modulated the antioxidant activity and reduce the oxidative stress and genotoxicity by quenching and detoxifying the free radicals induced by chlorsan against male reproductive disorder.

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