Protective Effect of Vitamin E and Selenium Combination on Cypermethrin-Induced Toxicity in Male Rats

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ABSTRACT:
The present study was designed to investigate the adverse effects of Cypermethrin (CYP) intoxication on the liver, kidney, brain and heart and the probable alleviating capability of vitamin E and Selenium combination (VE/Se) against such effects. Twenty-four male albino rats were divided randomly into 4 equal groups (6 rats each). CYP-treated rats received CYP (4.67 mg/kg bwt equals 1/10 LD50) orally once daily. CYP +VE/Se-treated rats were injected subcutaneously with 0.2 ml/kg bwt Viteselen®15 twice weekly with concurrent daily administration of CYP once daily. VE/Se-treated rats and control group. After 60 days treatment all rats were euthanized. CYP induced significant increase in serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), urea, creatinine and malondialdehyde (MDA) levels and a significant reduction in reduced glutathione (GSH) level. Also, CYP revealed marked degenerative and necrotic alterations in liver, kidneys, brain and heart. Conversely, treatment with VE/Se combination improved CYP-induced oxidative damage and the histopathological alterations of these organs. Results indicated that CYP exerts significant harmful effects. And the concurrent administration of VE/Se partly reduced these detrimental effects.

1. INTRODUCTION:
Pyrethroid insecticides represent a major class of very effective multipurpose insecticides, accounting for about 30% of global insecticide markets (Bhushan et al., 2013). Newly designed analogues have been synthesized and launched periodically, boosted up with enhanced potential against pre-existing pests, and those that have become resistant (El-Magd et al., 2011 and Adjrah et al., 2013). They are structurally similar to pyrethrins, a class of compounds that are found in chrysanthemum plants, and become an integral component of various pest eradication programs as insecticides for modern farming, in agricultural settings, gardens, and industrial areas and for the control of ectoparasites and vector borne diseases of domestic, laboratory animals (e.g., lice and fleas) in sheep, cats, dogs, poultry and other farm animals and household pests (Assayed et al., 2010 and Wei et al., 2012). Cypermethrin (CYP), a synthetic type II pyrethroid insecticide has been extensively used in the last two decades in many of the developing countries, especially Saudi Arabia and Egypt (Sakr, 2003 and Singleton et al., 2014). Pyrethroids are considered as comparatively safe pesticides, but the increased uses of them have introduced serious, novel hazards to the human beings and their livestock (Addy-Orduna et al., 2011) which has necessitated accurate identification of their potential hazards (Saoudi et al., 2011). The World Health Organization classified CYP as moderately harmful, class II (WHO, 1995) as it was thought that CYP has low mammalian toxicity however, studies have shown that clinical, occupational, or environmental prolonged exposure to CYP particularly in developing countries causes chronic or persistent health hazards including cases of severe acute and chronic human and animal toxicity as hematological disorders (Sharaf et al., 2010), nephrotoxicity (Nair et al., 2011), hepatotoxicity (Bhushan et al., 2013) and reproductive toxicity (Fang et al., 2013). Also, it induced gastrointestinal (Nair et al., 2011) and neurological disturbances (Sankar et al., 2012). Therefore, this study was carried out to evaluate the pathological lesions besides the corresponding biochemical changes in response to the oxidative stress induced by daily oral intubation of CYP to male albino rats up to 60 days and the possibility of VE/Se to ameliorate this toxic effects.

2. MATERIALS AND METHODS
2.1. Animals
Seventy-two apparently healthy adult male Albino rats (140 – 160 g bwt 10 weeks age), were purchased from a closed random bred colony at the Medical Research Institute of Alexandria University,
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Egypt. They were housed under recommended environmental conditions and the basal diet up to two weeks before the experiment for adaptation. Rats were received humane care in compliance with the guidelines of the National Institutes of Health (NIH) of Animal Care and the local committee approved this study.

2.2. Estimation of lethal dose 50 (LD₅₀) of CYP.

Forty-two rats were orally administered CYP (cyperkill® 25 %EC), manufactured by CHIMACE-AGRIPHAR S.A. Belgium Company with different 7 concentrations (35, 40, 45, 50, 55, 60 and 65 mg/kg bwt). Six rats were kept as control group throughout the entire experimental period (7 days) administered 2 ml distilled water (vehicle of CYP) orally using stomach tube. Mortality was assessed and counted in the different groups. LD₅₀ was calculated according to the Kerber formula (Pershin, 1971).

2.3. Evaluation of the protective effects of Viteselen® 15 on CYP-induced-oxidative damage.

Twenty-four male albino rats were divided randomly into 4 equal groups (6 rats each), CYP-treated rats received CYP (4.67 mg/kg bwt equals 1/10 (LD₅₀) orally once daily using stomach tube, CYP+VE/Se-treated rats were injected subcutaneously with 0.2 ml/kg bwt Viteselen® 15 (contains 1.67mg sodium selenite plus 150 mg VE/ml, obtained from Adwia Co. S.A.E.) twice weekly using insulin syringe where the dose was calculated according to Paget and Barnes (1964) with concurrent daily administration of CYP (4.67 mg/kg bwt) once daily, VE/Se-treated rats were injected subcutaneously with 0.2 ml/kg bwt. Viteselen® 15. Control rats were subcutaneously injected with 2 ml/kg B.W. saline (vehicle of Viteselen® 15) twice weekly and was daily administered 2 ml distilled water (vehicle of CYP) orally. Rats in each group were euthanized after 60 days from the beginning of the experiment by cervical dislocation. Dead rats were necropsied immediately and examined grossly.

2.3. Biochemical analysis.

Blood samples were collected from the medial canthus of the eye before euthanasia in a plain centrifuge tubes. The resultant serum was stored in a clean and dry serum storage vial at -20 C° for estimation of AST and ALT activities (kits from Bio-labo, France) according to Reitman and Frankle (1957), Blood urea according the method of Tabacco et al. (1979) and serum creatinine according the method of Fabiny and Ertingshausen (1971) using commercial diagnostic kits supplied by Vitro Scient. Co. Egypt while analysis of reduced GSH and MDA were performed according to method of Sedlack and Lindsay (1968) and Placer et al., (1966) respectively, using kits supplied by Bio-diagnostic co. Egypt.

2.4. Histopathologic studies

Following necropsy, tissue specimens were collected from liver, kidneys, brain and heart and rapidly fixed in 10% neutral buffered formalin solution for at least 24 hrs. The fixed specimens were processed through the conventional paraffin embedding technique. Paraffin blocks were prepared from which 5 microns thick sections were obtained. These sections were stained with Hematoxyline and Eosin (H&E) according to the method described by Bancroft et al., (2013).

2.5. Statistical analysis

The analysis of variance (ANOVA) for the obtained data was performed using statistical analysis system (SAS, 2004) software to assess significant difference with the aid of Duncan test.

3. RESULTS

2.1. Clinical signs and mortalities:

CYP-treated rats showed depression and loss of appetite. The mortality percent was 33.3% while CYP+ VE/Se-treated rats showed mild anorexia and there were no mortalities observed. Neither significant clinical signs nor mortalities were recorded in VE/Se-treated rats and control rats throughout the period of the experiment.

Effect on body weight:

As shown in (Table1) daily administration of CYP and CYP+ VE/Se to male albino rats provoked a significant decrease of the body weight in all intoxicated groups as compared to VE/Se-treated and control rats. However, the reduction was less pronounced in the group treated with CYP + VE/Se.

Serum biochemical assays:

As recorded in (Table1) CYP and CYP+ VE/Se treated rats showed a significant increase in serum ALT, AST, urea, creatinine and MDA levels and a significant decrease in serum GSH level as compared to VE/Se-treated and control rats and the greatest effects were observed in the CYP treated group.
Table 1: Effects of Cypermethrin (CYP) and its combination with Viteselen (CYP+ VE/Se) on the body weights and biochemical parameters in male albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CYP</th>
<th>CYP + VE/Se</th>
<th>VE/Se</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>107.50±3.10 c</td>
<td>142.50±5.20 b</td>
<td>211.25±4.27 a</td>
<td>210.00±2.04 a</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>72.43±0.94 a</td>
<td>48.28±1.19 b</td>
<td>15.15±0.85 c</td>
<td>14.94±0.37 c</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>138.90±1.91 a</td>
<td>102.79±1.93 b</td>
<td>54.02±1.52 c</td>
<td>53.44±0.80 c</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>97.47±1.55 a</td>
<td>68.15±0.93 b</td>
<td>34.52±0.71 c</td>
<td>33.75±0.18 c</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.99±0.07 a</td>
<td>0.79±0.00 b</td>
<td>0.63±0.01 c</td>
<td>0.62±0.01 c</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>5.81±0.99 c</td>
<td>9.68±0.51 a</td>
<td>13.57±1.40 a</td>
<td>13.71±0.72 a</td>
</tr>
<tr>
<td>MDA (nmol/dl)</td>
<td>26.48±1.64 a</td>
<td>17.59±3.02 b</td>
<td>5.15±0.36 c</td>
<td>5.12±0.99 c</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±S.E. Values with different letters at the same raw are significantly different at P≤0.05 (ANOVA) with Duncan’s multiple range test. ALT= Alanine aminotransferase. AST =Aspartate aminotransferase. GSH=Reduced glutathione. MDA= Malondialdehyde.

3.2. Pathological results

3.2.a. Gross lesions:
CYP-treated rats showed hemothorax and pale enlarged liver and kidneys showed marked cortical atrophy while CYP+ VE/Se-treated rats showed only slightly enlarged liver with pale color. No apparent lesions were recorded in VE/Se-treated rats and control rats.

3.2.b. Histopathologic examination:

Livers of CYP-treated rats showed severe cytoplasmic vacuolation of the hepatocytes that appeared at the periportal areas (Fig.1, a). Multifocal areas of periportal hepatic and mid-zonal necrosis with mononuclear cells infiltration (Fig. 1, b) were present. Also, widespread hemorrhages and severe vascular congestion were noticed (Fig. 1, c). Furthermore, there was extensive brooding of the portal areas by connective tissue proliferation with the formation of newly formed bile ducts as well as intense mononuclear cell infiltration mainly lymphocytes and plasma cells(Fig. 1,d). Moreover, CYP+ VE/Se treated rats lesions were almost identical to those reported in CYP treated rats such as mild hepatocytic vacuolation (Fig. 1, e). The hepatocellular necrosis was less frequent characterized by few mononuclear cells infiltration with presence of regenerative oval cells (Fig.1, f). In addition, the portal areas showed mild mononuclear cell infiltration. Conversely, livers of VE/Se-treated and control rats had normal histological structure. Concerning the renal lesions More than 70% of examined renal tubules of CYP-treated rats showed severe tubular changes. The degenerated tubular epithelium showed severe vacuolation and some tubules showed tubular cystic dilatation. Necrotic tubules contain dark eosinophilic necrotic debris (Fig. 2, a).

Regarding the interstitium, interstitial nephritis with severe inflammatory cells infiltration mainly lymphocytes, plasma cells and macrophages and slight fibroplasia were widespread present around the necrotic tubules (Fig. 2, b) beside congestion of the intertubular blood vessels. The glomerular lesions were in the form of compressed capillary tufts with widening of Bowman’s space leading to increased urinary spaces (Fig. 2, c). Also, the glomeruli were necrotic with presence of necrotic remnant of capillary tufts. However, co-administration of CYP +VE to rats ameliorated these alterations, as the tubular necrosis was less frequent and the percentage of the affected glomeruli was significantly decreased in comparison with CYP- treated rat (Fig. 2, d). The renal tissues of VE treated control rats showed normal histological limits. On the other hand, the cerebrum of CYP-treated rats showed degenerated shrunken, hypereosinophilic and pyknotic neurons associated with satellitosis, and neuronophagia (Fig. 3, a) in addition to diffuse congestion in the cerebral blood vessels. Marked hemorrhages were seen in neocortex (Fig. 3, b) as well as lymphoplasmacytic perivascular cuffing (Fig. 3, c). Severe neuropil spongiosis with diffuse gliosis at the cerebral medulla were evident. The meninges were thickened by congested blood vessels and moderate lymphoplasmacytic cell infiltration. Conversely, Mild lesions were seen in the cerebrum of CYP+ VE-treated rats as slight gliosis and mild congestion of the cerebral blood vessels (Fig. 3, d). The cerebellum of CYP and CYP+VE/Se treated rats was almost normal as did VE/Se and control rats. Concerning the heart of CYP treated rats; the sarcoplasm of degenerated myocytes exhibited numerous variably sized discrete empty vacuoles fatty vacuoles. Multifocal necrosis of myocardium was evident associated with mononuclear cells infiltration (Fig. 4, a). As mentioned previously, the heart of
CYP+ VE/Se treated rats showed focal necrotic myositis with mild inflammatory cells infiltrates was present (Fig. 4, b). However, the necrotic foci were smaller in size and less frequent as compared to CYP- treated rats. Focal sarcoplasmic vacuolation mostly of lipid type was also noticed. However, heart of VE/Se-treated and control rats had normal histological limits.

Figure (1): Photomicrograph of liver from treated rats stained by H&E. Liver of CYP-treated rats (a) Hepatocytic vacuolation (arrows) X=100; (b) Mid-zonal necrosis with mononuclear cells infiltration (arrows) X=400; (c) Periportal necrosis (A), hemorrhages (black arrow) and mononuclear cells infiltration in portal areas (blue arrow). X=100 (d) Brooding of the portal areas by connective tissue proliferation (A) with formation of newly formed bile ducts (arrows). (e) X=100 Liver of CYP+VE/Se-treated rats showing mild hepatocytic vacuolation (arrows) and Slightly normal portal area (A) X=100; (f) Hepatocellular necrosis with few mononuclear cells infiltration and presence of regenerative oval cells (arrow) X=400.
Figure (2): Photomicrograph of kidney from treated rats stained by H&E. Kidney of CYP-treated rats showing (a) Necrotic renal tubules contain dark eosinophilic necrotic debris (arrows) X=100. (b) Interstitial nephritis with severe mononuclear cells infiltration(A) X=400. (c) Atrophied glomerular capillary tufts with widening of Bowman’s space (black arrow) necrotic glomeruli (blue arrows) with complete absence of some glomerular capillary tufts(arrow heads) X=100. (d) Kidney of CYP+VE/Se-treated rats showing tubular necrosis(A) with necrotic glomeruli(arrow ) X=100.

Figure (3): Photomicrograph of brain from treated rats stained by H&E. Brain of CYP-treated rats showing (a) Degenerated, hypereosinophilic and pyknotic neurons associated with satellitosis, and neuronophagia (arrows) with diffuse gliosis(A) X=400. (b) Marked hemorrhages in neocortex (arrows) X=100 (c) Lymphoplasmacytic perivascular cuffing (arrows) X=400. (d) Brain of CYP+VE/Se-treated rats showing slight gliosis(black arrow) and mild congestion of the cerebral blood vessels(blue arrow) X=100.
4. DISCUSSION

In the present study administration of CYP to male rats led to anorexia, depression and decreased feed intake. Mortalities were observed only in CYP–treated rats. The cause of death was likely related to sever hepatic and renal lesions concurrently with the severe general weakness and anorexia observed particularly in dead rats. Administration of CYP provoked a significant decrease of the body weight in all intoxicated rats as compared to control groups in accordance with Islam and Hoque (2015). This may be attributed to the effect of CYP on gastrointestinal tract resulting in decreased appetite and impaired absorption of nutrients from gut or might be due to direct toxicity of CYP (Sankar et al., 2012). Also, CYP induced a significant increase in the mean values of ALT, AST, urea and creatinine levels as compared to VE/Se treated and control rats. The elevations in these markers are correlated with hepatic and renal dysfunction. These findings were in agreement with Nair et al. (2011), Bhushan et al. (2013) and Amir et al. (2015). CYP is expected to have two modes of action: due to its lipophilic nature it easily crosses and accumulates in biological membranes leading to stimulate the production of (ROS) and result in oxidative damage to essential cell components caused by oxygen-free radicals (Fetoui et al., 2008). Also, previous studies suggested that some direct effects related to CYP toxicity could be due to CYP accumulates in cell membrane and changes in membrane structure and function (Mansour and Mossa, 2010). Thus, the increase in lipid peroxidation and depletion of antioxidant enzymes play a major role in CYP-induced toxicity. Consequently, CYP-treated rats showed significant decrease in GSH and increase in MDA levels as compared to VE/Se treated and control rats. The same results were reported by Kaur and Dar (2013) and Sharma et al. (2014). In the same way, the most pronounced CYP-induced hepatic lesions of the present study were cellular vacuolation, peri-portal and mid-zonal hepatocellular necrosis hemorrhages and portal fibrosis with intense mononuclear cell infiltration in portal areas. These lesions were in harmony with those reported by Aslam et al., (2010). CYP metabolized in the liver via the hydrolytic ester cleavage and with oxidative pathway by the cytochrome P-450 microsomal enzyme system, caused oxidative stress by reducing the activity of superoxide dismutase and glycogen level, leading to hepatic degeneration and necrosis (Khan et al., 2009). Periportal necrosis is characteristic for most toxins and hepatocytes surrounding the portal triads are vulnerable to necrosis because they are the first cells exposed to the toxins meanwhile, the hepatocyte in centrilobular area not affected in part because they are farthest from incoming arterial and portal blood containing toxins. Also, the centrilobular hepatocyte contain the greatest concentration of cytochrome P450 which in part responsible for biotransformation of CYP into inactive less toxic metabolites than their parents (Heder et al., 2001). The mentioned hepatic lesions were parallel to those reported by Grewal et al., (2010), Nair et al., (2011), Aslam et al., (2010) and Islam and Hoque (2015). However, none of these previous reports described the pattern of hepatic necrosis in details and their pathological description was briefed and short. The renal lesions in CYP-intoxicated rats revealed tubular vacuolation and necrosis, interstitial leukocytic infiltrations, congestion of renal blood vessels and glomerular atrophy, congeston and necrosis. Sakr and Al-barakai
(2014) and Islam and Hoque (2015) recorded similar renal lesions. These lesions could be attributed to CYP-induced oxidative damage. On the other hand, the cerebrum of CYP-intoxicated rats showed individual neuronal degeneration and necrosis, perivascular lymphocytic cuffs, gliosis, neuronal vacuolation, associated with congestion in the meninges. These lesions were in harmony with those reported by Sayim et al. (2005), Muthuviveganandavel et al., (2008) and Grewal et al., (2010). Many pesticide agents are reported to cause variable changes in the brain on repeated exposure, which have been related to hypoxia, hypoglycemia, and/or damage to cell ion homeostasis (Grewal et al., 2010). Moreover, CYP crosses the blood-brain barrier and induces neurotoxicity and motor deficits (Ahmad et al., 2009). It prolongs the opening of sodium channel, a major site of its action, leading to hyper-excitation of the central nervous system (Trainer et al., 1997), modulates chloride, voltage-gated calcium and potassium channels, (Ray and Fry, 2006), alters the activity of glutamate and acetylcholine receptors and ATP, induces DNA damage and oxidative stress in the neuronal cells (Sharaf et al., 2010). CYP also, modulates the level of neurotransmitters, including GABA and dopamine (Manna et al., 2005). The lesions of myocardium included mild foci of lymphocytic myocarditis associated with myocyte vacuolation. Clinically, it was correlated with serum AST elevation. Grewal et al., (2010) reported congestion and haemorrhagic foci, with loss of cross striations and eosinophilic appearance of cardiomyocytes in CYP-intoxicated crossbred calves. Nair et al., (2011) reported haemorrhage and congestion with focal myolysis in CYP treated rats. Previously, it had been reported that VE is protective against pesticide-induced toxicity (Guney et al., 2007, Al-Othman et al., 2011 and Al-Shaikh, 2013). VE is a naturally occurring antioxidant nutrient and a lipid soluble vitamin present in lipid bilayer membranes that plays an important role in animal health by inactivating harmful free radicals and inhibits free radical formation (Kalender et al., 2004). Also, it showed protective effect on some biochemical indices induced by CYP toxicity (Altuntas and Delibas, 2002). Moreover, Se is an essential element for normal growth. The metabolic role of Se in mammalian cell occurs due to its function in the active site of seleno-enzyme glutathione peroxidase (Akhtar et al., 2009). Se is an essential cofactor of glutathione peroxidase (GSH-Px), the body’s master antioxidant that detoxifies H2O2 and organic peroxides and plays an important role in the protection of tissue from oxidative damage owing to pesticide toxicity. Aside from being an integral part of GSH-Px, Se can antagonize the toxic effects of some chemical substances and protects normal cell function by supporting the body’s natural defenses and scavenging harmful free radicals (Raneva et al., 2002). VE and Se are not only effective against oxidative damage alone, but also have a synergistic effect when used in combination (Aslam et al., 2010). The absorption of VE is impaired by severe Se deficiencies and Se alleviates VE deficiencies by allowing higher levels of VE to be absorbed (Sharaf et al., 2010). In this study co-treatment of male rats with CYP+VE/Se revealed a significant improvement in the body weight, decreased serum transaminases, urea and creatinine levels, reduction in LPO and as a consequence improvement in GSH level as compared to CYP-treated rats and the improvement in the histological structure of liver, kidney, brain and heart was correlated to the potential role of VE and Se in scavenging ROS generated by CYP. In conclusion, this study demonstrated the detrimental effect of CYP in male albino rats. Moreover, the concurrent administration of VE/Se partly alleviated these effects.

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