Amelioration of Chlorpyrifos Induced Retinal and Renal Toxicity by Vitamin D₃

1Ghada Ghanem El-Hossary, 2Amal Ahmed El-Gohary, 3Nadia Samy Ahmed, 4Anisa Saleh Mohamed and 4Sahar Mahmoud Mansour

1Pharmacology, 2Physiology, 3Nutrition and 4Histology Departments, Research Institute of Ophthalmology, Giza, Egypt.

Abstract: Vitamin D is a multipurpose steroid hormone and can reduce the neurotoxicity produced by a variety of toxicants. The present investigation is an attempt to ameliorate the toxicity of chlorpyrifos by using 1 alpha, 25 dihydroxy vitamin D₃ (1, 25 VD). Twenty four male Wistar rats were used and were divided into four groups (six rats each). Groups I and II were the negative and positive controls respectively. In group III, chlorpyrifos was administered once orally by a stomach tube in a dose of 63 mg/kg on the first day of experiment (untreated chlorpyrifos toxicity). In group IV, in addition to chlorpyrifos, animals were treated with IM injection of 1.35 mg/kg 1, 25 VD on day 1 and on day 15 of the experiment. Rats were subjected to examination by Electroretinogram (ERG) on days 15 and 30 of the experiment. In each group, two animals were sacrificed on day 15 and four animals on day 30. Retinal and kidney specimens were collected and subjected to light microscopic examination. Chlorpyrifos toxicity model (group III) showed a significant deterioration of ERG parameter values (a-wave and b-wave amplitudes and b-wave latency) as well as obvious histopathological changes in both renal and retinal tissues. Treatment with 1, 25 VD injections (group IV) showed significant improvement of ERG and histopathological examinations as compared to untreated toxicity model. In conclusion, 1, 25 VD can be markedly protective to renal and retinal tissues against chlorpyrifos induced toxic changes.

Key words: Chlorpyrifos, Retinal and Renal Toxicity, neuroprotective vitamin D₃, electroretinography.

INTRODUCTION

Chlorpyrifos is an organophosphate insecticide that is widely used in agriculture, industry and household. Neurotoxicity is one of its main toxic manifestations and can occur after long term exposure to subclinical doses of chlorpyrifos as well as after acute intoxication (Sanchez-Santed et al., 2004). In addition, increasing evidence from animal and human studies indicates that chlorpyrifos is a developmental neurotoxicant (Tait et al., 2009). The main mechanism of acute intoxication is due to inhibition of acetylcholinesterase which is responsible for the degradation of the neurotransmitter acetylcholine. The resultant cholinergic overactivity leads to the manifestations of neurotoxicity (Karanth and Pope, 2000).

Several other mechanisms have been implicated in producing the neurotoxicity of chlorpyrifos and these mechanisms are unrelated to its anticholinesterase action. In the mouse retina, chlorpyrifos caused increased cell apoptosis, lipid peroxidation and DNA damage accompanied by decreased activities of several antioxidant enzymes (Yu et al., 2008). The same mechanisms operate in the kidney as chlorpyrifos can cause nephrotoxicity due to increased lipid peroxidation and decreased antioxidant potential by increasing oxidative stress (Oncu et al., 2001). Studies conducted on indoor environments after using chlorpyrifos have shown that exposure to this toxicant can occur from inhalation of residual air concentrations and from oral or dermal exposure from residues on floors, carpets, children toys, food and dust (Lemus and Abdelghani, 2000). Due to this unavoidable exposure to chlorpyrifos, particularly in agricultural areas, it is important to investigate drugs that have the potential to protect against organophosphate toxicity in different tissues and at the same time these drugs should be cheap and available.

Vitamin D is a multipurpose steroid hormone with several functions in the nervous system including neuroprotection (Cherniack et al., 2009). It is activated in the liver and kidney leading to the production of the active form; 1 alpha, 25 dihydroxy vitamin D₃ (1, 25 VD) (Wardlaw and Smith, 2006). Having a strong
potential for neuroprotection, 1, 25 VD can reduce the neurotoxicity produced by a variety of toxicants (Li et al., 2008). The neuroprotective effects of 1, 25 VD can be produced by a variety of mechanisms including prevention of oxidative injuries and apoptosis (Lin et al., 2003). It also possesses anti-inflammatory, anti-proliferative and anti-angiogenic effects which make it a good agent for protecting various tissues against a variety of pathological conditions including toxic agents (Taverna et al., 2005).

In a previous study in our laboratory, it was reported that chlorpyrifos produced retinal damage and nephrotoxicity by inducing oxidative stress and apoptosis (El-Shazly et al., 2009). Since both mechanisms can be antagonized by vitamin D, therefore, the present study is designed to investigate the possible protective effects of vitamin D, on chlorpyrifos-induced retinal toxicity and nephrotoxicity in albino rats. The animals are subjected to examination by electroretinogram (ERG) as well as histopathological examination of the retinal and renal tissues.

MATERIALS AND METHODS

Induction of Chlorpyrifos Acute Toxicity:
Chlorpyrifos (Chlorofet from Vapco, Jordan) was reconstituted in corn oil to get a 1% concentration to be administered in a dose of 63 mg/kg as a single oral dose by a stomach tube (Bebe and Panemanglore, 2003).

Animals:
Twenty four male Wistar rats weighing 140-150 grams, aged 3 months, were used. They were individually housed in separate cages under standardized temperature (25-28°C), humidity (50%-60%) and light conditions (12 hours light–dark cycles). They were fed the standard diet and water for 30 days. All care and handling were in accordance with institutional guidelines for use of animals in ophthalmic and vision research with approval of the institutional authority for laboratory animal care.

Animals were divided into four groups; each was consisting of six rats. Group I- Animals received an equivalent volume of saline by IM injections on day 1 and on day 15 (negative control). Group II- Animals were treated with 1.35 mg/kg vitamin D₃ (Devarol-S from Memphis, Egypt) by IM injection on day 1 (the same day of chlorpyrifos administration) and on day 15. The dose was extrapolated from the human therapeutic dose according to Paget and Barnes (Paget and Barnes, 1964) (positive control). Group III- Chlorpyrifos was administered once orally by a stomach tube in a dose of 63 mg/kg on the first day of experiment (untreated chlorpyrifos toxicity). Group IV- Chlorpyrifos was administered as mentioned above and the animals were treated concomitantly with IM injection of 1.35 mg/kg vitamin D₃ on day 1 and on day 15 of the experiment. On day 15, before receiving the second injection in groups I, II and IV, two animals were sacrificed from each group and subjected to histopathological examination. The remaining four animals in each group remained until day 30 and received the stated treatments.

Electrophysiological Tests:
Electroretinogram (ERG) using the Italian EREV 99 system (for recording and analysis by averaging) was performed before the experiment to establish baseline standards and on days 15 and 30 from the beginning of the experiment. The rats were dark adapted for at least 30 min after pupillary dilatation. Then, rats were anesthetized using a single intraperitoneal injection of thiopental sodium (Thiopental from Sandoz, Austria) in a dose of 40 mg/kg. The active electrode was placed near the margin of the lower eyelid; the reference electrode was placed on the forehead and the earth electrode was clipped to the earlobe. Recording of combined response was carried out using white flash stimulus having frequency of 1 flash/second and energy of 1 joule, with 0dB background intensity. Amplitudes were measured from baseline to the lowest point of the negative peak for the a-wave and from the latter (or baseline, if absent) to the positive peak for the b-wave. In addition, the a- and b-wave peak latencies were calculated from the flash onset to the peaks of the waves.

Histological Examination:
After the final ERG tests, rats were anesthetized by ether and sacrificed by cervical dislocation. The eyes were immediately enucleated, bisected and placed in 2.5 % glutaraldehyde fixative for six hours. After a buffer wash, retinas were dissected, post fixed in 1% osmium tetroxide, dehydrated in a series of graded ethanol and embedded in Araldite CY 212. Semithin sections were cut and stained with toluidine blue (TB). Specimens of kidney tissue were also collected, processed for paraffin sections and stained by haematoxyline and eosin (Hx&E). Slides were examined by Olympus light microscope and photographed by Olympus camera.
Statistical Analysis:
Amplitudes of a- and b-waves (microvolt; μV) and b-wave latency (millisecond; ms) of ERG were expressed as the mean±SD. Analysis of variance (ANOVA) and student t test were performed to compare the responses between groups. A post-hoc test was used to isolate significant differences if (P < 0.05).

RESULTS AND DISCUSSIONS

Result:
Electrophysiological Tests:
At the first day of the study, ERG recordings (a-wave amplitude, b-wave amplitude and b-wave latency) showed no significant differences between the negative control group and the other groups (P>0.05). In addition, there were no significant differences in these ERG parameters when comparing the positive control group (group II) with negative control group (group I) after 15 and 30 days (Tables 1 and 2). Chlorpyrifos toxicity model (group III) showed a marked reduction of ERG parameter values (a-wave and b-wave amplitudes) and a marked increase in b-wave latency with a significant difference as compared to the negative control group after 15 days (Table 1 and Fig. 1). Amplitudes of a- and b-waves showed more significant reduction after 30 days of toxicity in comparison with the negative control group. However, the b-wave latency did not deteriorate any further after 30 days and there was insignificant change when compared to group I (Table 2 and Fig. 1).

Treating chlorpyrifos toxicity with vitamin D (group IV) prevented the marked deterioration in the ERG parameters. After 15 days, the a-wave and b-wave amplitudes were significantly improved as compared to the untreated toxicity model (group III), but the b-wave latency did not improve and was still insignificantly different as compared to group III. The ERG parameters didn’t return to normal values after 15 days with a significant difference from control group I (Table 1 and Fig. 1). The treating effect of vitamin D was obvious after 30 days as there was a significant improvement in all ERG parameters in treated chlorpyrifos toxicity (group IV) as compared to untreated toxicity (group III). Furthermore, all ERG mean values were back to normal with no significant difference found when compared to the negative control group (Table 2 and Fig. 1).

It is worth mentioning that a-wave latency was insignificantly changed in all the study groups, so it was not included in the tabulated results.

Table 1: Mean values (±SD) of a- & b-wave amplitudes and b-wave latency after 15 days in group I (negative controls), group II (positive controls), group III (untreated chlorpyrifos toxicity) and group IV (chlorpyrifos toxicity treated by vitamin D).

<table>
<thead>
<tr>
<th>ERG Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-wave amplitude (μV)</td>
<td>4.46±0.83</td>
<td>4.53±0.58</td>
<td>1.30±1.53</td>
<td>3.19±0.77</td>
</tr>
<tr>
<td>P₁</td>
<td>0.822</td>
<td>0.017*</td>
<td>0.042*</td>
<td>0.036*</td>
</tr>
<tr>
<td>P₂</td>
<td>0.000*</td>
<td>0.013*</td>
<td>0.006*</td>
<td>0.013*</td>
</tr>
<tr>
<td>b-wave amplitude (μV)</td>
<td>14.35±0.84</td>
<td>14.39±1.01</td>
<td>7.38±1.70</td>
<td>11.07±1.46</td>
</tr>
<tr>
<td>P₁</td>
<td>0.921</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>P₂</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>b-wave latency (ms)</td>
<td>53.05±10.54</td>
<td>52.32±10.15</td>
<td>75.17±5.53</td>
<td>69.71±10.23</td>
</tr>
<tr>
<td>P₁</td>
<td>0.907</td>
<td>0.011*</td>
<td>0.000*</td>
<td>0.020*</td>
</tr>
<tr>
<td>P₂</td>
<td>0.327</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD, n=6; μV=microvolt; ms=millisecond ;*significant difference.

P₁ value: compared to group I; P₂ value: compared to group III.

Table 2: Mean values (±SD) of a- & b-wave amplitudes and b-wave latency after 30 days in group I (negative controls), group II (positive controls), group III (untreated chlorpyrifos toxicity) and group IV (chlorpyrifos toxicity treated by vitamin D).

<table>
<thead>
<tr>
<th>ERG Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-wave amplitude (μV)</td>
<td>4.44±0.50</td>
<td>4.20±0.32</td>
<td>0.31±0.37</td>
<td>4.56±0.60</td>
</tr>
<tr>
<td>P₁</td>
<td>0.436</td>
<td>0.000*</td>
<td>0.042*</td>
<td>0.000*</td>
</tr>
<tr>
<td>P₂</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>b-wave amplitude (μV)</td>
<td>14.47±1.10</td>
<td>14.15±1.06</td>
<td>5.49±0.98</td>
<td>14.16±1.04</td>
</tr>
<tr>
<td>P₁</td>
<td>0.721</td>
<td>0.001*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>P₂</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>b-wave latency (ms)</td>
<td>53.85±12.39</td>
<td>54.98±11.93</td>
<td>73.88±5.21</td>
<td>52.38±9.07</td>
</tr>
<tr>
<td>P₁</td>
<td>0.684</td>
<td>0.051</td>
<td>0.9</td>
<td>0.040*</td>
</tr>
<tr>
<td>P₂</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD, n=4; μV=microvolt; ms=millisecond ;*significant difference; P₁ value: compared to group I; P₂ value: compared to group III.
Fig. 1: ERG recordings of selected rabbit eyes in groups I (negative control), III (untreated chlorpyrifos toxicity) and IV (chlorpyrifos toxicity treated by vitamin D₃ injection). ERG combined response demonstrated markedly reduced a- and b-wave amplitudes and delayed b-wave latency in group III. After 15 days in group IV, the a- and b-wave amplitudes were improved while the b-wave latency was still delayed. The ERG parameters returned to normal values after 30 days.

**Histological Examination:**
Histological examination of the negative control group I showed normal appearance of both renal and retinal tissues in specimens obtained after 15 and 30 days of the experiment. The kidney cortex examination revealed that cortical parenchyma consisted of renal corpuscles together with proximal and distal convoluted tubules. Each glomerulus was formed of a glomerular tuft of capillaries surrounded by Bowman's capsule (Fig. 2). The control retina exhibited nine layers representing the neural retina and the supporting retinal
Fig. 2: Light micrograph of control kidney cortex (group I) showing that most of cortical parenchyma surrounding renal corpuscles (c) are consisting mainly of proximal and smaller numbers of distal convoluted tubules (Hx & E X500).

pigment epithelium (Fig. 3). After 15 and 30 days of the experiment, the positive control group II showed normal appearance of both renal and retinal tissues with no apparent deviation from the negative control group I. After 15 days of chlorpyrifos induced toxicity (group III), light microscopic examination of the kidney showed shrunken glomeruli surrounded by wide Bowman's space. Extravasated blood elements were seen obviously in the cortical parenchyma. Moreover, the nuclei of epithelial cells lining the tubules were pyknotic. Interstitial edema was clearly seen (Fig. 4).

Fig. 3: Light micrograph of control retina (group I) showing the supporting retinal pigment epithelium (Pe) and the normal nine retinal layers: 1- photoreceptors, 2- outer limiting membrane, 3- outer nuclear layer, 4- outer plexiform layer, 5- inner nuclear layer, 6- inner plexiform layer, 7- ganglion cell layer, 8- nerve fibre layer and 9- inner limiting membrane (TB X500).
Fig. 4: Light micrograph of kidney cortex of group III (untreated chlorpyrifos toxicity) showing shrunken renal glomeruli (g) surrounded by wide Bowman's space. Extravasated blood elements (e) are seen in the cortical parenchyma. Nuclei of epithelial cells lining renal tubules are pyknotic. Interstitial edema (I) is clearly obvious (Hx & E X500).

Retinal examination after 15 days of untreated chlorpyrifos toxicity (group III) showed vacuolated retinal pigment epithelium. The outer segments of photoreceptors were distorted and dislodged. The Muller cell processes encircling the outer and inner nuclear layers were edematous (Fig. 5). Moreover, the retinal astrocytes showed nuclear pyknosis (Fig. 6). After 30 days of chlorpyrifos induced toxicity (group III), light microscopic examination of both renal and retinal tissues exhibited approximately the same findings and severity of changes as those seen after 15 days of intoxication. In group IV (chlorpyrifos toxicity treated by vitamin D₃), the kidney cortex obtained after 15 days revealed residual edema of Bowman’s capsule, glomerular tuft as well as the epithelial cells of renal tubules (Fig. 7). The retinal specimens obtained at the same period displayed mild fragmentation of outer segments of photoreceptors. A striking observation was the absence of edema of supportive cells (Fig. 8). After 30 days in group IV, light microscopic examination of all kidney specimens showed normal architecture of renal corpuscles and tubules (Fig. 9). In addition, examination all retinal specimens showed that all retinal layers displayed no deviation from controls (Fig. 10).

Fig. 5: Light micrograph of retina of group III (untreated chlorpyrifos toxicity) showing vacuolated retinal pigment epithelium (v). The outer segments of photoreceptors are distorted and dislodged. The Muller cell processes (p) encircling the outer and inner nuclear layers are edematous (TB X1250).
Fig. 6: Light micrograph of retina of group III (untreated chlorpyrifos toxicity) showing pyknosis of glial cells (PG) (TB X1250).

Fig. 7: Light micrograph of kidney cortex obtained after 15 days in group IV (chlorpyrifos toxicity treated by vitamin D$_3$ injection) showing residual edema of Bowman’s capsule (b), glomerular tuft of blood capillaries (g) as well as in epithelial cells of renal tubules (Hx & E X500).

Fig. 8: Light micrograph of retina obtained after 15 days in group IV (chlorpyrifos toxicity treated by vitamin D$_3$ injection) showing mild fragmentation of outer segments of photoreceptors. No edema of supportive cells can be seen (TB X500).
Fig. 9: Light micrograph of kidney cortex obtained after 30 days in group IV (chlorpyrifos toxicity treated by vitamin D$_3$ injection) showing normal architecture of renal corpuscles and tubules (Hx & E X500).

Fig. 10: Light micrograph of retina obtained after 30 days in group IV (chlorpyrifos toxicity treated by vitamin D$_3$ injection) showing no deviation from normal control group in all retinal layers (TB X500).

**Discussion:**

Chlorpyrifos is an extensively used organophosphate insecticide having many urban and agricultural crop pest control uses. The present study is an attempt to protect against Chlorpyrifos-induced retinotoxicity and nephrotoxicity by using 1, 25 VD injections in albino rats.

In the present investigation, Chlorpyrifos produced disturbances of the ERG parameters (decrease in a- and b-wave amplitudes and increase in b-wave latency). These disturbances were evident after 15 and 30 days of intoxication. These results are in agreement with previous investigations which reported that acute exposure of rats to chlorpyrifos produced decrease in a- and b-wave amplitudes (Yoshikawa et al., 1990a) as well as increase in a- and b-wave latencies (Yoshikawa et al., 1990b). On the other hand, chronic exposure of rats to chlorpyrifos (1 or 5 mg/kg/day for one year) with or without oral spike doses of 45 mg/kg every two months produced no significant difference in a- and b-wave amplitudes which doesn't agree with our results (Geller et al., 2005). However, our ERG measurements were done 15 and 30 days after chlorpyrifos intoxication. Geller and associates (Geller et al., 2005) performed the ERG measurements 3-5 months after completion of dosing which may give enough time for the retina to recover from the toxic effects of chlorpyrifos.
In the present study, chlorpyrifos produced histopathological changes in both retina and kidney. These changes were evident after 15 days and persisted after 30 days of intoxication. Although ERG disturbances were more severe after 30 days than after 15 days of chlorpyrifos toxicity, morphological changes were not. Specimens of retina and kidney obtained 15 and 30 days after chlorpyrifos toxicity showed nearly the same picture and severity of histopathological changes. In agreement with the results of the present investigation, El-shazly and colleagues (El-Shazly et al., 2009) reported approximately the same histopathological changes in both retinal and kidney tissues obtained seven days after acute intoxication with chlorpyrifos. In addition, Oncu et al. (2001) reported the histopathological picture of chlorpyrifos nephrotoxicity in the form of infiltration by mononuclear cells, hydropic degeneration in the epithelium of the tubules and glomerular sclerosis which also coincide with our results.

Chlorpyrifos was also reported to affect rat glial cells in vitro. There was cytotoxicity and inhibition of glial cell proliferation particularly astrocytes (Guizzetti et al., 2005). This also agrees with our results which revealed pyknotic changes in retinal astrocytes in rats intoxicated with chlorpyrifos. Moreover, it was reported that acute chlorpyrifos intoxication increase cell apoptosis, lipid peroxidation and DNA damage in mouse retina (Yu et al., 2008) which reflects changes in retinal structure and function that coincides with the results of the present investigation. The histopathological changes and the ERG disturbances are interrelated. The present investigation revealed histopathological changes in the photoreceptor segment which was distorted and dislodged. This correlates with a-wave disturbance as the a-wave arises from the light evoked closure of sodium channels along the outer segment plasma membrane of the photoreceptor cells and it reflects photoreceptor function (Robson and Fishman, 1996).

The present study also revealed that glial cells of the retina are one of the targets of chlorpyrifos toxicity. Chlorpyrifos affected Muller cells representing the glial population of the neural retina. The histopathological changes were in the form of edema of Muller cell processes which extended from the inner to the outer limiting membranes. These processes completely envelope the neural elements. This can lead to impaired function of Muller cells and lack of structural and metabolic support to the retinal neurons. The histopathological changes of Muller cell processes might also interfere with its important role in terminating synaptic transmission by active uptake of neurotransmitters. This also correlates with b-wave disturbances of ERG as the b-wave results from the current flow along Muller cells in response to increase in extracellular potassium ion concentration (Robson and Fishman, 1996). Another glial cell influenced by chlorpyrifos toxicity is the astrocyte which showed pyknosis of its nuclei. This endangers the retina as the main function of retinal astrocytes is segregation of the receptive surface of neurons from extraneous influences (Wheater, 2002).

In the present investigation, 1, 25 VD could protect the retina and kidney tissues against the toxic effects of chlorpyrifos. Attempts to antagonize or protect against the toxic effects of chlorpyrifos using 1, 25 VD were not found in the literature to compare our results. However, 1, 25 VD was reported to be protective to the kidney in chronic renal disease (Dusilova-Sulkpva, 2009) and it could suppress podocyte apoptosis which is an early step in the pathophysiological progression to proteinuria and glomerulosclerosis (Xiao et al., 2009). In addition, 1, 25 VD was reported to protect the retina against N-methyl-D-aspartate-induced retinal cell damage in mice by induction of metallothionein which is an endogenous antioxidant (Suemori et al., 2006). Similarly, 1, 25 VD is expected to protect against chlorpyrifos-induced retinal and renal toxicity which is concluded in the present investigation.

There are some suggested mechanisms by which 1, 25 VD can antagonize the toxic effects of chlorpyrifos. Chlorpyrifos produces its toxic effects not only through cholinesterase inhibition but also by inducing oxidative stress and apoptosis. This toxicant was reported to produce marked deterioration of oxidative stress biomarkers; glutathione, antioxidant enzymes and antioxidant potential in rats (Guizetkin et al., 2001). In addition, Qiao and coworkers (Qiao et al., 2005) proved that oxidative mechanisms were contributing to the developmental neurotoxicity of chlorpyrifos. Moreover, Giordano and colleagues (Giordano et al., 2007) showed that chlorpyrifos toxicity involve the generation of reactive oxygen species.

Regarding apoptosis, organophosphates were reported to induce apoptosis in immune cells through a direct effect on mitochondria which finally leads to DNA damage and cellular death (Li and Kawada, 2006). Chlorpyrifos was also reported to induce mitotic abnormalities and dose dependent apoptosis in cultured rat embryos (Roy et al., 1998).

Yu and coworkers (Yu et al., 2008) reported both oxidative stress and apoptosis induced by chlorpyrifos. They reported decreased activity of antioxidant enzymes together with increased lipid peroxidation, DNA damage and apoptosis in retina of mice acutely intoxicated with chlorpyrifos. They also stated that oxidative stress can induce apoptosis and therefore, protection against oxidative stress can protect the cells against apoptosis (Yu et al., 2008).
Vitamin D was reported to protect different types of cells against oxidative stress and apoptosis. It was reported to protect the rat brain against zinc-induced oxidative stress and apoptosis (Lin, et al., 2003). In addition, 1, 25 VD could protect human prostate epithelial cells against oxidative stress and DNA damage (Bao et al., 2008). Moreover, 1, 25 VD prevented puromycin aminonucleoside-induced apoptosis of glomerular podocytes and therefore protect against renal damage (Xiao et al., 2009).

Heat shock protein (hsp) is a sensitive integrative indicator of stress, such as oxidative stress, and tissue damage. Its expression is increased in response to a variety of stressors including chlorpyrifos toxicity (Eder et al., 2007). In fact, hsp can be a sensitive indicator to even sublethal doses of chlorpyrifos. Expression of hsp can be increased in muscle and liver of salmon fish intoxicated by chlorpyrifos (Eder et al., 2009) as well as in brain and liver of chlorpyrifos intoxicated rats (Bagchi et al., 1996).

Heat shock protein-32 was significantly decreased by a combination of 1, 25 VD and 17-beta estradiol in rat brains lesioned by photothrombosis which can be an additional prove to the antioxidant effects of 1, 25 VD owing to the fact that hsp-32 is a well established marker of cerebral oxidative stress response (Losem-Heinrichs et al., 2004).

Chlorpyrifos toxicity is characterized by loss of mitochondrial potential which leads finally to activation of caspases; the main enzymes responsible for development of apoptosis (Li and Kawada, 2006). Vitamin D, was reported to stabilize mitochondrial potential and decrease apoptotic biomarkers in rat cortical cells intoxicated by cyanide (Li, et al., 2008). This can be an additional mechanism conferring the neuroprotective and antiapoptotic roles of 1, 25 VD.

In conclusion, 1, 25 VD can be markedly protective to renal and retinal tissues against chlorpyrifos induced toxic changes. Several mechanisms can be suggested such as the antioxidant and antiapoptotic effects of 1, 25 VD. Further investigations are required to prove these suggested mechanisms and to study the role of 1, 25 VD against other neurotoxicants.

REFERENCES


