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Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: The protective role of Vitamin E

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Abstract

Diazinon (DZN) is an organophosphate insecticide has been used in agriculture and domestic for several years. Vitamin E (200 mg/kg, twice a week), diazinon (10 mg/kg, per day) and Vitamin E (200 mg/kg, twice a week) + diazinon (10 mg/kg, per day) combination were given to rats orally via gavage for 7 weeks. Pseudocholinesterase in serum and haematological indices were investigated at the end of the 1st, 4th and 7th weeks comparatively with control group. At the end of 1st, 4th and 7th weeks, statistically significant decrease of pseudocholinesterase activity in serum were detected when diazinon- and Vitamin E + diazinon-treated groups compared to control group. When diazinon- and Vitamin E + diazinon-treated groups were compared to each other there were no significant changes. When diazinon-treated group was compared to control group, body weight decreased significantly at the end of the 4th and 7th weeks. It was observed that at the end of 1st, 4th and 7th weeks, there was a statistically significance in haematological indices except mean corpuscular hemoglobin (MCH) when diazinon-treated group was compared to control group. At the end of 1st week increase of thrombocyte, at the end of the 4th week increase of hemoglobin and thrombocyte and at the end of the 7th week increase of red blood cell (RBC), hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC) and thrombocyte were observed statistically significant when Vitamin E + diazinon treated group was compared with diazinon treated group. According to the present study, we conclude that Vitamin E reduces diazinon toxicity, but it does not protect completely.

Keywords: Organophosphate insecticides; Diazinon; Vitamin E; Pseudocholinesterase; Haematology

1. Introduction

Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution and therefore, are a cause of concern. Organophosphorus (OP) compounds are a major component of many pesticides with widespread use in both agricultural and domestic situations. These compounds or their active metabolites inhibit acetylcholinesterase (AChE) and are therefore acutely toxic due to accumulation of acetylcholine at cholinergic synapses leading to increased activation of nicotinic and muscarinic receptors. Various types of delayed toxicity may also occur (de Blaquiere et al., 2000). Pseudocholinesterase is similar in structure to AChE, but it is encoded by a separate gene, and is synthesized primarily in the liver and is found in plasma and other tissues (Mehrani, 2004). Toxicities of OP pesticides cause adverse effects on many organs and systems (Joshi et al., 2003; Neishabouri et al., 2004; Kalender et al., 2005a).

Diazinon (0,0-diethyl-0-[2-isopropyl-6-methyl-4-pyrimidinyl]phosphorothioate) is an OP insecticide with a broad range of activity which inhibits acetylcholinesterase activity. It has been widely and effectively used throughout the world with applications in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruit and vegetables and as a pesticide in domestic and agricultural (Garfitt et al., 2002). Diazinon causes changes in liver enzymes and biochemical indices and swelling of mitochondria in hepatocytes (Kalender et al., 2005a). Also diazinon causes toxic effects on blood cells, spleen, thymus and lymph nodes of rats (Handy et al., 2002). Meanwhile diazinon causes toxic effects on other organisms (Svoboda et al., 2001).

Many insecticides are hydrophobic molecules, which bind extensively to biological membranes, especially to the phospholipids bilayers (Lee et al., 1991). Vitamin E (α-

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tocopherol) is a family of lipid-soluble vitamins and acts as an antioxidant in cells, interrupting the propagation of lipid peroxidation in the plasma membrane and thus preserving membrane integrity (Warren et al., 2000). Studies carried out with antioxidant such as α-tocopherol, have shown that they inhibit free radical formation (Kalender et al., 2004, 2005b) and may effectively minimize lipid peroxidation in biological systems (Kalender et al., 2002; Gokalp et al., 2005). It was reported that Vitamin E decreased diazinon hepatotoxicity and protected some of the biochemical indices (Kalender et al., 2005a).

The aim of this study was to investigate the effects of diazinon on pseudocholinesterase activity and haematological indices and protective effect of Vitamin E in rats.

2. Material and methods

2.1. Animals

Male Wistar rats (weighting approximately 230–250 g) obtained from the Refik Saydam Central Hygiene Institute, Ankara, Turkey were used. The animals were housed in plastic cages, fed a standard laboratory diet and water ad libitum. Rats were exposed to a 12-h light:12-h dark cycle, at a room temperature of 18–22 °C. Animals were quarantined for 10 days before being randomized into experimental groups of three animals per cage.

2.2. Chemical

Diazinon, purity 99%, was obtained from Agricultural Struggle Center, Ankara, Turkey. Vitamin E (DL-α-tocopherol acetate) was supplied by Merck (Germany).

2.3. Animal treatment schedule

Rats were divided into two groups, control ($n = 24$) and experiment groups ($n = 72$). Rats in experiment group were divided into three groups, Vitamin E treatment group ($n = 24$), diazinon treatment group ($n = 24$) and Vitamin E + diazinon treatment group ($n = 24$). At the end of the 1st, 4th and 7th week, eight rats were dissected in each group, blood and tissue samples were taken for biochemical and ultrastructural investigations.

The substances were administered in the morning (between 09:00 and 10:00 h) to non-fasted rats. All rats were treated for 7 weeks. First exposure day to diazinon was accepted experimental day 1. Vitamin E was given alone and together with diazinon 1st and 4th day of each experimental week.

2.3.1. Control group

Corn oil at a dose of 10 mg/kg per day was given through gavage to rats once a day.

2.3.2. Vitamin E-treated group

Vitamin E (200 mg/kg) was administered orally through gavage twice a week.

2.3.3. Diazinon-treated group

Diazinon at a dose of 10 mg/kg per day in corn oil was given through gavage to rats once a day.

2.3.4. Vitamin E + diazinon-treated group

Vitamin E (200 mg/kg, twice a week) was administered orally through gavage 15 min before oral administration of diazinon (10 mg/kg per day, once a day in corn oil) through gavage.

2.4. Measurement of body weight

Body weight of control and treated rats were measured at the end of the 1st, 4th and 7th weeks by automatic balance (AND GX-600, Japan)

2.5. Evaluation of pseudocholinesterase activity

At the end of the 1st, 4th and 7th weeks rats were anaesthetized by diethylether, blood samples were taken from hearts to the sterile tubes. Blood samples were centrifuged at 3500 rpm for 20 min for pseudocholinesterase, and serum was separated. The analysis of pseudocholinesterase enzyme activity is performed in Roche Hitachi 912 analyzer using Roche cholinesterase kit (ChE, EC 3.1.1.8). This assay is based on the method published by Knedel and Böttger (1967). The principle of this method is colorimetric assay. Based on this assay, samples were added phosphate buffer (60.7 mmol/l, pH 7.7) containing 5,5′-dithiobisnitrobenzoic acid (DTNB) as chromogen. As substrate butyrylthiocholine iodide (43.5 mmol/l) was used. Cholinesterase catalyzes the hydrolysis of butyrylthiocholine iodide to thiocholine iodide and butyrate. Thiocholine iodide reacts with 5,5′-dithiobisnitrobenzoic acid to form the yellow product 2-nitro-5-mercaptobenzoate. The rate of formation of 2-nitro-5-mercaptobenzoate is proportional to the ChE activity and is measured photometrically.

2.6. Evaluation of haematological parameters

Blood samples with anti-coagulant EDTA were analyzed for haematological parameters [red blood cell (RBC) counts, hemoglobin, hematocrit, white blood cell (WBC) counts, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and thrombocytes] using Bayer-Advia 120 hematology analyzer.

2.7. Statistical analysis

Data were analyzed using SPSS 11.0 for windows. The significance was calculated using one-way analysis of variance (ANOVA) and followed by Tukey multiple comparison procedure to calculate the significance. $P < 0.01$ value was taken as statistically significant.

3. Results

3.1. Evaluation of body weight

No death was observed in any of the experimental groups. No significant differences were observed in body weight between Vitamin E-treated group and the control group during experimental period. No statistically significant changes were detected in body weight when diazinon- and Vitamin E + diazinon-treated group compared with control group at the end of 1st week. Fourth and seventh weeks after in the diazinon- and Vitamin E + diazinon-treated groups, body weight was significantly decreased compared to control group ($P < 0.01$). When Vitamin E + diazinon-treated group was compared to diazinon-treated group, no statistically significant change was observed in body weight at the end of the 4th and 7th weeks (Fig. 1).

3.2. Pseudocholinesterase activity

No statistically significant changes were detected in pseudocholinesterase activity when Vitamin E-treated group compared with control group at the end of 1st, 4th and 7th weeks. A significant decrease was observed in pseudocholinesterase activity at the end of 1st, 4th and 7th weeks in diazinon- and Vitamin E + diazinon-treated group compared with control group