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Research Article

The Potential of Emamectin Benzoate to Induce Kidney DNA Oxidation, Heat Shock Protein Levels and Apoptosis in Male Mice

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Abstract

Biopesticide Emamectin benzoate (EMB), which is used in a wide range of areas such as food production and household pest removal, causes toxic effects caused by different uses and can cause health problems in human life. As a result of pesticide exposure, it causes biological damage such as oxidative toxicity and initiation of apoptosis signal and genotoxic effects. In sublethal EMB doses; E1 (25 mg/kg/day), E2 (50 mg/kg/day), E3 (100 mg/kg/day) were administered to Swiss albino male mice for 14 days by gavage. Male mice in kidney tissues; changes in thiobarbituric acid reagent (TBARS) levels, HSP70 (heat shock proteins 70) levels known as stress protein, apoptosis marker caspase 3 enzyme activity and DNA oxidation marker 8-hydroxy-2-deoxyguanosine (8-OHdG) levels were investigated. The results of this study showed that the increase in TBARS level in kidney tissue after exposure to EMB caused lipid peroxidation. An increase in HSP70 levels were determined due to the stress response resulting from the toxicity of the tissues of the EMB groups. In addition, increases in caspase 3 enzyme activation and 8-OHdG levels were determined to cause both apoptosis and DNA oxidation. These changes in different molecular toxicology parameters indicate that the toxicity of EMB is effective in the kidneys of male mice at 14 days of exposure.

Keywords: Emamectin Benzoate; Caspase 3; HSP70; 8-OhdG; Mice; Kidney

Introduction

Pesticides are man-made chemicals designed to control organisms such as harmful insects, weeds and fungi in agriculture and households. they can also reach non-target organisms, such as humans [1]. Pesticides are biologically active substances with a toxicity specific to the chemical structure. It is considered to be a common toxic source that causes health problems in humans. Exposure to pesticides has a different incidence of chronic diseases in humans and may also lead to accelerated disease processes in pesticide-related toxicities [2,3]. Emamectin benzoate (EMB) is a natural toxin of 4'-deoxy-4'-epimethyl-amino benzoate of naturally occurring avermectins [4]. Avermectins are used in the treatment of external and internal parasites in humans and animals, including parasitic nematodes that cause human diseases; onchocerciasis and lymphatic filariasis [5]. The toxicity of pesticides is usually demonstrated by the production of reactive oxygen species (ROS). In pesticide exposure, the potential for ROS formation increases, resulting in oxidative stress [6]. Antioxidant defense systems that detoxify ROS include a number of antioxidative enzymes and low molecular weight non-enzymatic antioxidants. They are systems that protect biological systems against environmental and chemical stress at the molecular level [7]. Many studies have shown an association between increased ROS production and cell death (apoptosis). Heat shock protein (HSP), also known as stress protein, is induced by increased oxidative stress formation with increased ROS. Under normal conditions, HSP70 proteins function as ATP-dependent molecular chaperones that assists in folding, transporting and assembling the proteins of the cells as the most conserved type of HSP in vertebrates [8]. Caspases (cysteine-aspartic acid proteases) are an important enzymatic element of programmed apoptosis. Among them, caspase-3 is a death protease that is frequently activated and catalyzes the specific cleavage of many important cellular proteins. Caspase-3 is important for normal cell development and constitutes a distinctly important and essential pathway against a stimulating tissue, cell type and death stimulus in other apoptotic scenarios. Caspase-3 is required in the normal apoptosis process and is involved in the completion of the process

for apoptotic chromatin condensation and DNA fragmentation in all cell types examined [9]. Caspase-3 is activated by both external (death ligand) and internal (mitochondrial) pathways in apoptotic cells [10]. 8-hydroxy-2-deoxyguanosine (8-OHdG) is an important biomarker in the determination of genotoxic effects that show the occurrence and severity of DNA oxidation. 8-OHdG is an end product of oxidative damage caused by specific enzymatic degradation in the 8-hydroxylation of guanine base. 8-hydroxy-2-deoxyguanosine (8-OHdG) is an important biomarker in the determination of genotoxic effects indicating the formation and severity of DNA oxidation [11]. Damage caused by oxidative damage to DNA has been reported to be biologically important. The role of reactive oxygen species (ROS) in the production of DNA single strand breaks is well known [12].

Materials and Methods

Chemicals

Emamectin benzoate formulation sold as commercial Uman Proclaim Opti UV 5 was WGA was purchased from Syngenta distributor in Turkey. The chemicals used were obtained from Sigma Aldrich and Merck.

Test animals

Experimental animals are 10 weeks old male Swiss albino male mice (25-30 gr) in the laboratories of the Experimental Medical Research and Application Center of the Çukurova University Faculty of Medicine (ÇUTF-DETAUM). The animals were acclimatized in their cages for 5 days with a suitable humidity and temperature ratio for 12 hours day and night. All albino mice were fed ad libitum with standard laboratory pellet feed and tap water and no special diet was applied.

Experimental design

Albino mice were found to be 8 mice in each group, and 4 groups were formed together with the control group. The control group (C) was administered same amount of water under the same experimental conditions and by oral gavage. Experimental groups [13] Wolterink., *et al.* (2012) determined by the LD_{50} value; E1 group (25 mg/kg/day = 1/30 LD_{50}), E2 group (50 mg/kg/day = 1/15 LD_{50}), E3 group (100 mg/kg/day = 1/7.5 LD_{50}) administration daily oral gavage. The toxicity study was continued for 14 days. The animals were sacrificed by cervical dislocation under mild ether anesthesia. Heart tissues were frozen at -80ºC for use until biochemical analysis.

Biochemical analysis

Stored kidney tissue samples were divided into 2 parts, the first part was homogenized in PBS (phosphate buffered saline solution) containing 2.5 mM ATP for 3 minutes at 10000 rpm for 20 minutes at at 4ºC for 20 minutes at 16000g. supernatant fractions were separated by centrifugation. In the supernatant; protein, TBARS, 8-OHdG and HSP70 levels were determined. The second part of the kidney tissue was separated for caspase 3 enzyme activity measurement and homogenized for 1 minute at 10000 rpm with Ultra-Turrax homogenizer with special buffer 1/10 (w/v) in the kit. The homogenates were centrifuged at 13000 xg for 15 min. at +4ºC.

- **Determination of Total Protein Level:** The supernatants and bradford reagent were mixed and allowed to stand at room temperature for 15 minutes. As standard, bovine serum albumin (BSA) was read against the standard values prepared in the microplate reader at a wavelength of 595 nm, the results compared with the calculation of mg/ml [14].
- **HSP70 Level:** The non-competative enzyme-linked immunosorbent assay (ELISA) method measures the absorbance of the orange colored product at 492 nm in 2 replicates for each sample in a microplate reader [15].
- **TBARS Level:** TBARS, a secondary product of lipid peroxidation, was a lipid peroxidation product as a biomarker. It was measured by spectrophotometric method at a wavelength of 532 nm, indicating damage in lipid metabolism [16].
- **Activity of Caspase-3 Enzyme:** Caspase-3 enzyme activity was determined by the method specified in the caspase-3 assay kit produced by Sigma commercial company.
- **8-OHdG Level:** DNA oxidation measurement was measured at a wavelength of 450 nm by the competitive ELISA method [17].
- **Statistical Analysis:** Statistical analysis of biochemical analysis data were performed using the One Way Anova-Duncan test ($P < 0.05$) in the SPSS 22.0 (SPSS Inc., Chicago, IL) package program.

Result and Discussion

In this study, we found a 15%, 36% and 55% increase in heart HSP70 level (Figure 1), 14-day exposure in E1, E2 and E3 groups, respectively. Increases in TBARS levels; The E1, E2 and E3 groups were 52%, 90% and 142% respectively (Figure 1, $P < 0.05$). The reason for the overproduction of HSP70 levels in renal tissue has been associated with cytoprotection in various renal epithelial cell lines HSPs play a role in the regulation of cellular damage by acting as molecular chaperones for damaged proteins [18]. No change in HSP60 or HSP70 levels was detected in male Wistar rats, brain tissue in Malathion exposure [19]. A dose-dependent increase in hsp70 levels in the liver of rats was determined with chlorpyrifos exposure at different doses [20].

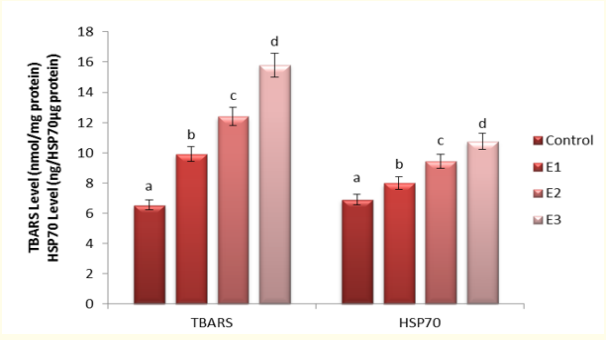

Figure 1: HSP70 level (ng/HSP70µg protein) and TBARS level (nmol/mg protein) in kidney of Swiss albino mice after exposure to sublethal doses of EMB for 14d.The letters a, b, c and d indicate differences between concentrations. Data showing different letters are significantly different at the p <0.05 level (N =8).

TBARS is the most abundant individual aldehyde structure resulting from lipid peroxidation. TBARS level is an important predictor of lipid peroxidation [21]. Fipronil (2.5, 5 and 10 mg/kg bw) significantly increases TBARS levels in liver, kidney, and brain tissues in 28 organs for 28 days in male mice under Fipronil exposure, and is a common information in Fipronil-induced organ toxicity [22,23]. ROS may cause membrane dysfunction due to oxidation of polyunsaturated lipids [24]. Fipronil showed that TBARS levels in liver tissue in male albino rats increased significantly and stimulated lipid peroxidation [25]. In contrast to this study, oral exposure to low atrazine doses (0.3 mg/kg or 12.5 mg/kg) in rat liver and kidney did not alter TBARS levels [26].

In this study, caspase 3 enzyme activities (Figure 2) were increased in E1, E2 and E3 groups by 47%, 83% and 133% respectively. 8-OHdG levels were significantly increased in E1, E2 and E3 groups by 26%, 68% and 102% (Fig. 2, P <0.05).Organophosphorus insecticide phorate 14 day exposure to different oral doses of male wistar rats tissues increased caspase 3 gene expression and also found a significant increase in lipid peroxidation [27]. A significant increase in caspase 3 and 9 enzyme activities was determined in phorate exposed rats. These enzymes show increased activity in the apoptotic pathway with increased toxicity. Previous studies of pesticides and organic pollutants have also increased activation of caspase 9 and 3 [28,29]. Furthermore, the increase in enzyme activity of caspase 3 is thought to be irreversible in the apoptotic signaling cascade [30]. Caspases are considered to be a central player for and during the apoptotic process [31]. In particular, caspase-3 plays an important role in the initiation of apoptosis signals. In the last stage of apoptosis induces a large number of morphological changes in the characteristics of cells entering apoptosis [32].

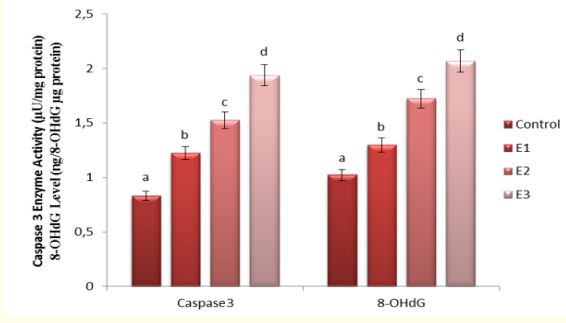

Figure 2: Caspase 3 enzyme activity (µU/mg protein) and 8-OHdG level (ng/8-OHdG µg protein) in kidney of Swiss albino mice after exposure to sublethal doses of EMB for 14d. The letters a, b, c and d indicate differences between concentrations. Data showing different letters are significantly different at the p <0.05 level (N =8).

In the cell, ros attack results in oxidative stress and ROS, which reaches the DNA structure, attacks the guanine base and induces its hydroxylation. As a result, 8-OHdG is formed and DNA fractures are formed. In the normal repair mechanism, 8-hydroxyl-guanine-DNA glycosidase (hOGG1) removes 8-OHdG from double-stranded DNA. 8-OHdG can be transported into the blood and disposed of as urine from the kidney [33]. Increased levels of 8-OHdG were detected in groups exposed to pesticide DDT (dichlorodiphenyltrichloroethane) that developed hepatic carcinoma. Increased levels of 8-OHdG may play an important role in the development of DDT in hepatocarcinogenesis. Because 8-OHdG leads to base mismatch (mutation) on DNA replication, DNA is thought to be due to damage to hepatocellular DNA [34]. At 300 mg/kg exposure to glyphosate, male mice showed no change in 8-OHdG levels in the tissues of the kidneys, whereas Roundup showed a significant increase in DNA oxidative damage at 900 mg/kg exposure [35].

Conclusion

In conclusion, this study showed that increased TBARS levels in EMB exposure caused lipid peroxidation in kidney tissue of albino male mice. Increased caspase-3 enzyme activity shows that apoptosis pathway is formed as a result of toxicity. As a result of toxicity caused by EMB, HSP70 is known to increase its level due to insufficient antioxidant system. However, it is thought that there is not enough response in cell protection. It was determined that EMB may have genotoxic effect and increased DNA oxidation biomarker

8-OHdG level. In this study, it was found that EMB shows toxicity on human and ecological health by important biochemical and genotoxic parameters.

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