



Protective effect of *Funalia trogii* crude extract on deltamethrin-induced oxidative stress in rats

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ARTICLE INFO

Article history:

Received 15 March 2010

Received in revised form 28 September 2010

Accepted 3 October 2010

Keywords:

Funalia trogii

Cold buffer extract

Deltamethrin

Antioxidant enzymes

Liver enzymes

ABSTRACT

In this study the protective effects of cold buffer extract of *Funalia trogii* ATCC 200800 (FtE) and vitamin E (VitE) on oxidative stress induced with deltamethrin using oral administration in rats were investigated. Deltamethrin treatment caused an increase in liver enzyme activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) ($p < 0.05$); however, it caused a decrease in activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRd) when compared to control group ($p < 0.05$). Activities of AST, ALT, ALP enzymes and level of thiobarbituric acid reactive substances (TBARS) decreased significantly after VitE administration ($p < 0.05$). Both enzyme activities and TBARS levels were found similar in VitE and FtE treated rats shortly after pesticide administration ($p < 0.05$). In conclusion, it appears that FtE prepared in cold buffer has capability to prevent the liver damage like VitE against the toxic effect of deltamethrin.

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1. Introduction

Deltamethrin, a synthetic pyrethroid type II, is globally used in crop protection and control of malaria and other Vector-borne diseases. The main sources of general population exposure to this pesticide are contaminated food and water, deltamethrin is readily absorbed by the oral route (Barlow, Sullivan, & Lines, 2001). Several studies have demonstrated the genotoxic and immunotoxic effects of deltamethrin in mammalian species (Lukowicz-Ratajczak & Krechniak, 1992). Induction of oxidative stress is one of the main mechanisms of deltamethrin toxicity (Tuzmen, Candan, Kaya, & Demiryas, 2008; Yousef, Awad, & Mohamed, 2006).

Reactive oxygen species (ROS) are constantly formed as by-product of normal metabolic reactions and their formation is accelerated by accidental exposure to occupational chemicals like pesticides. In healthy individuals, the generation of reactive oxygen species is well balanced by the counterbalancing act of antioxidant defences. Hence an imbalance between ROS generation and antioxidant status has been described as oxidative stress (Marubayashi, Dohi, Ochi, & Kawasaki, 1985). The cellular antioxidant pool com-

prises of integral antioxidants like glutathione and other thiols and antioxidant free radical scavenging enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd). Long-term oxidative stress is also expressed as changes in extent of lipid peroxidation (Yu, 1994).

Vitamin E is a naturally occurring antioxidant nutrient that plays an important role in animal health by inactivating harmful free radicals produced through normal cellular activity and various stressors. Also vitamin E inhibits free radical formation for the protection of cells against oxidative damage due to pesticides toxicity (El-Demerdash, Yousef, Kedwany, & Baghdadi, 2004). Yousef et al. (2006) found that deltamethrin (1.28 mg/kg BW) exposure of rats resulted in free radical-mediated liver damage, as indicated and hepatic lipid peroxidation, which was prevented by vitamin E (100 mg/kg BW).

Mushrooms have long been appreciated for their flavour and texture as vegetables and also they provide a wealth of protein (~22%), fibre (~63%), fat (~5%), vitamins (thiamin, riboflavin, niacin, and biotin), and minerals (~10%) (Mattila, Sounpa, & Piironen, 2000). Moreover, mushrooms could accumulate a variety of secondary metabolites including phenolics, steroids, terpenes, polypeptides. The antioxidative and free radical scavenging properties of phenolics of mushroom extracts have been reported (Mau, Chang, Huang, & Chen, 2004). Hot water extract of *Lentinus edodes*

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and basidiomycetes-X was also found to have high antioxidant activity against lipid peroxidation (Cheung & Cheung 2005). *Funalia trogii* ATCC 200800 has been reported to degrade xenobiotic compounds such as azo, heterocyclic, and reactive dyes (Ozsoy, Unyayar, & Mazmanlı, 2005). Antitumor activity of *F. trogii* extract was also showed on mammalian cells (Unyayar et al., 2006).

In an earlier study it was demonstrated the protective role of *F. trogii* on lipid peroxidation generated by deltamethrin in liver, by monitoring the chances of MDA concentration and by electron microscopy observation (Balli et al., 2009). The aim of this study was to investigate the protective effect of cold buffer extract of *F. trogii* ATCC 200800, grown on wheat bran and soybean flour, on deltamethrin-induced oxidative stress in rats. The protective effect of the extract was also compared with that of a well known antioxidant, vitamin E.

2. Materials and methods

2.1. Chemicals

A commercial formulation of the pyrethroid insecticide deltamethrin, [IUPAC name: (S)-alpha-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate and CA name: 1R-[1_(S),3_]3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid, cyano(3-phenoxyphenyl)methyl ester] DECIS 2.5 EC (deltamethrin 25 g L⁻¹-Bayer) was used. Vitamin E (Dietvit® E, 53% α -tocopherol acetate) was purchased from Merck (Germany). Other chemicals and reagents were purchased from Sigma–Aldrich Chemical Co. and Merck. Wheat bran and soybean flour were supplied from a farm land.

2.2. Preparation of *F. trogii* extracts (FtE)

F. trogii culture was obtained from Environmental Biotechnology Laboratory, Environmental Engineering Department, University of Mersin, Turkey. The fungus was maintained on Potato Dextrose Agar (PDA; Merck) slants and incubated at 30 °C for 5 days and stored at 4 °C.

The solid-substrate fermentation (SSF) medium used for producing the fungal biomass consisted of wheat bran and soybean flour (90:10). The substrate was humidified with a 0.1 M, pH 6.0 sodium phosphate buffer (added at 60% v/w). The humidified medium was placed in 1-L Erlenmeyer flasks and autoclaved at 120 °C for 60 min. The autoclaved medium was inoculated with the fungal stock cultures that had been grown on PDA. The flasks were incubated for 10 days at 30 °C. Then, the contents of the flasks were dried (Sanyo MIR 152 incubator) for 24 h at 40 °C. The dried material was ground in a coffee grinder for 2 min. The ground biomass powder (1 g) was suspended in potassium phosphate buffer (10 mL, 0.1 M, pH 6.0) for 15 min. Solids were removed by centrifugation at 12,000g (Hettich Micro 22R) for 15 min (Unyayar et al., 2006). The supernatant (extract) was sterilized using a 0.22 μ m filter and diluted with aforementioned phosphate buffer to desired concentrations. Hot water or alcohols were not used for extraction in any step of this work.

2.3. Specification of FtE

Cold buffer extract of FtE used in this research was analysed and summarised in Table 1. Laccase and peroxidase enzyme activity was determined using ABTS as a substrate. One unit of enzyme activity (U) was defined as the amount of enzyme that formed 1 μ mol ABTS per min. Protein concentration (expressed in mg mL⁻¹ FtE) was determined by a modified Lowry method (1951) with bovine serum albumin as a standard protein. Specific

activities (SA) of enzymes were calculated by dividing total enzyme activity to total protein (Unit/mg protein). The yield of extract was found 128 \pm 0.08 mg g⁻¹ of dry SSF media.

2.4. Preparation of deltamethrin and vitamin E (VitE)

The tested dose of deltamethrin was adjusted at 1.28 mg/kg BW (1/100 LD₅₀) in corn oil (Worthing, 1983). The dose 100 mg/kg BW of Vitamin E prepared with corn oil was used because previous studies showed that this dose was effective against the toxicity of deltamethrin (Yousef et al., 2006).

2.5. Animals treatment schedule

Twenty healthy adult male Swiss albino Wistar rats (6–8 weeks of age and average body weight 180–200 g) were used in this study. Rats were obtained from the Experimental Animal Centre, University of Mersin, Turkey. The study was approved by the research and ethical committee of the Mersin University. The rats were housed in polycarbonate boxes with steel wire tops and rice husk bedding. They were maintained in a controlled atmosphere of 12 h dark/light cycle, 22 \pm 2 °C temperature, and 50–70% humidity, with free access to pelleted feed and fresh tap water. After 2 weeks of acclimation, animals were randomly divided into four groups of six animals each. All groups were fed with standard pellets. Group I (control group) was orally given corn oil. Group II (deltamethrin group) was treated with deltamethrin alone. Group III (deltamethrin + VitE group) was treated with deltamethrin, after 30 min Vit E was given; Group IV (deltamethrin + *F. trogii* group) was treated with deltamethrin after 30 min FtE (50 mg/kg BW) was given. Rats were orally treated at 48 h intervals with repeated doses of deltamethrin, VitE and FtE, for 30 days. At the end of the experiments all rats were sacrificed by decapitation under ketamine (KETALAR-Eczacıbası) anaesthesia. Trunk blood samples were collected from the sacrificed animals placed immediately on ice. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 860g for 20 min. The liver was quickly excised, rinsed in ice-cold 0.175 M KCl/25 mM Tris–HCl (pH 7.4) and homogenised in glass–Teflon homogenizer (Heidolph S01 10R2R0). The liver homogenates were centrifuged at 10,000g for 15 min (Hettich U32) and the supernatants were used for antioxidant enzyme assays and lipid peroxidation determination in spectrophotometer (Perkin–Elmer Lambda EZ210).

2.6. Biochemical analysis

Plasma aspartate transaminase (AST; EC 2.6.1.1), alanine transaminase (ALT 2.6.1.2) and alkaline phosphatase (ALP 3.1.3.1) were assayed using kits approved by the IFCC. In liver SOD (EC 1.15.1.1) activity was measured at 505 nm and 37 °C and was calculated according to the inhibition percentage of formazon formation (McCord & Fridovich, 1969). The catalase (CAT; EC 1.11.1.6) assay, consisting of the spectrophotometric measurement of 10 mM hydrogen peroxide (H₂O₂) breakdown at 240 nm was assayed following the method of Beers and Sizer (1952). GPx catalyses the oxidation of GSH to GSSG by H₂O₂. The rate of GSSG formation was then measured by following a decrease in absorbance of the reaction mixture containing NADPH and glutathione reductase at 37 °C and 340 nm as NADPH is converted to NADP. *t*-Butyl hydroperoxide was used as a substrate. GRd (EC 1.8.1.7) activity was assayed by the oxidation of NADPH by GSSG at 37 °C and 340 nm (Beutler, 1984). GPx activity (EC 1.11.1.9) was determined according to the method of Beutler (1984). TBARS, as a marker for LPO, were measured at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA) (Ohkawa, Ohishi, & Tagi, 1979).

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