

Original article

Effect of albendazole anthelmintics on the enzyme activities of different tissue regions in *Eisenia fetida*

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Abstract

The purpose of this study was to determine the effect of albendazole (ABZ) on acid phosphatase (AP), glutathione S-transferase (GST) and Ca^{2+} -adenosine triphosphatase (Ca^{2+} -ATPase) activities in different tissue regions of earthworms and the whole earthworm after 2, 7 and 14 days exposure. The results were as follows: AP activities in the anterior and the mid-part were sensitive to ABZ. Particularly, AP activity in the anterior decreased significantly at concentrations between 100 mg kg^{-1} and 600 mg kg^{-1} after 14 days. GST and Ca^{2+} -ATPase activities in the mid-part were sensitive to ABZ, GST activity being inhibited significantly by ABZ concentrations between 400 mg kg^{-1} and 600 mg kg^{-1} after 7 days and at concentrations between 200 mg kg^{-1} and 600 mg kg^{-1} after 14 days. GST activity was negatively correlated with ABZ concentrations. Ca^{2+} -ATPase activity in the mid-part was inhibited significantly at concentrations between 200 mg kg^{-1} and 600 mg kg^{-1} after 7 days; after 14 days, Ca^{2+} -ATPase activity recovered to the control level. In conclusion, the different tissue regions of *Eisenia fetida* show different responses in enzymatic activities to ABZ and, therefore, provide early warning signals of soil contamination.

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1. Introduction

Veterinary drugs are often used to help maintain the health condition of domestic animals and ensure their well-being [1]. However, incorrect or excessive use in recent decades has caused substantial amounts of drugs and their metabolites to be released to agricultural land

through manure application. One potential adverse effect is harming some non-target organisms utilizing the excrement, and soft-bodied organisms exposed without orally consuming contaminated manure, such as earthworms, can be at risk if the potential toxins are leached from the manure into the pore water [14]. To date, only limited investigation has been conducted on the ecotoxicological effects of veterinary drugs [11,15].

Albendazole (ABZ), a derivative of benzimidazole, exhibits broad spectra of gastrointestinal anthelmintic activity and has been used widely in livestock. The

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anti-helminth mechanism of benzimidazole carbamate has been studied [5]. Previous report indicated that ABZ binds to β -tubulin and inhibits its polymerization to form microtubules [18]. ABZ also inhibits fumarate reductase and glucose transport, which is associated with adenosine triphosphate (ATP) synthesis [9]. Given orally by mammalian, ABZ is partially metabolized, with part of the absorbed drug is excreted unchanged in feces and urine. The main bioactive metabolite, alben-dazole-sulfoxide, an effective anthelmintic [5], is also excreted to the environment. However, no comparable ABZ metabolism and toxicity effects studies have hitherto been performed on soil animals such as earthworms.

Earthworms are preferred bioindicators to be used for assessing chemical contamination to soils [21]. As important species in decomposer communities, earthworms have a great impact on decomposition activity, nutrient mineralization and primary production. In particular, earthworms can perceive a diverse range of chemical stimuli and response through certain reactions accordingly [17]. Early biochemical reaction of earthworms on chemicals could provide important information in assessing potential adverse effects on soil environment. Enzymatic activities are regarded as fast and prognostic indices of individual reaction to the environmental stress, and should allow prediction of the consequences of pollution at population and ecosystem levels [17].

The main purpose of present study was to assess the biochemical responses of *Eisenia fetida* upon exposure to ABZ by measuring acid phosphatase (AP), glutathione S-transferase (GST) and Ca^{2+} -adenosine triphosphatase (Ca^{2+} -ATPase) activities in different tissue regions and the whole earthworm.

2. Materials and methods

2.1. Experimental procedures

E. fetida was obtained from an earthworm farm at China Agriculture University, kept in the test substrate (cattle manure) in the laboratory for a week prior to the start of the experiment. The individual earthworms were clitellate adults. ABZ-free cattle manure was used as test substrate in this study. ABZ (Sigma) with a purity >98% was first dissolved in acetone then mixed with manure to give nominal treatment concentrations of 0, 100, 200, 400 and 600 mg kg⁻¹ dry weight. Distilled water was added to the mixture to reach a 75% of weight increase. The substrates (pH 7.0 and C/N: 24) after being treated with ABZ were transferred to plastic containers, which were placed in ventilated cabinet to allow residual acetone to evaporate overnight. Water was added to

compensate for the lost weight due to acetone evaporation, and then earthworms were introduced. Each treatment group contains 12 containers and each container contains 12 earthworms (350 $\pm$ 20 mg each). The containers were maintained in an environmental chamber under 23 $\pm$ 1 °C and 12/12-h photoperiod.

2.2. Sample preparation and enzyme measurement

Prior to further examination and analysis after sampling, earthworms were incubated without feeding for 2 days on wet filter paper at room temperature to empty their gut content. The earthworm samples in four separate containers were collected at days 2, 7 and 14 and three enzymatic activities were measured in the anterior region (mouth to segment 25), mid-part (segments 25–33), posterior region (segment 33 to the anus) as well as in the whole earthworm. The whole earthworm or dissected parts from each container were pooled together, snap-frozen in liquid nitrogen and ground with liquid nitrogen, then homogenized in four parts (w:v) of Tris–HCl buffer (0.1 M, pH 7.6). Then a part of homogenate was centrifuged at 10,000 g for 10 min for AP and GST activities measurement, the remaining part of homogenate was centrifuged at 3000 g for 15 min for Ca^{2+} -ATPase measurement, and the supernatants were snap-frozen in liquid nitrogen and stored at –80 °C until analysis. All tissue preparations for enzyme were carried out at 4 °C.

AP activity was measured using the method from Hønsi and Stenersen [13] and GST activity was measured as described by Lukkari et al. [17]; ATPase activity was measured using ATPase detection kit from Nanjing Jiancheng Bioengineering Institute in China. Protein content was measured according to Bradford [2].

2.3. Statistical analysis

Parametric tests were preceded by tests for normal distribution. Differences in enzyme activity were tested for treatment significance using one-way analysis of variance (ANOVA). In addition, post hoc comparison (LSD test) was carried out to check for differences between the exposed and control groups. All statistics were performed using SPSS software (SPSS13.0).

3. Results

3.1. AP activity

AP activity in three tissue regions and the whole earthworm changed based on ABZ concentrations and

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