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Toxicology

# Antioxidant effect of selenium on lipid peroxidation, hyperlipidemia and biochemical parameters in rats exposed to diazinon

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ABSTRACT

Diazinon (DZN) is one of the most organophosphate insecticides that widely used in agriculture and industry. Selenium is generally recognized to be a trace mineral of great importance for human health, protecting the cells from the harmful effects of free radicals. Therefore, the present study was carried out to investigate the alterations in biochemical parameters, free radicals and enzyme activities induced by diazinon in male rat serum, and the role of selenium in alleviating the negative effects of DZN. Animals were divided into four groups of seven rats each; the first group was used as control. Groups 2, 3 and 4 were treated with selenium (Se; 200 μg/kg BW), diazinon (DZN; 10 mg/kg BW) and diazinon plus selenium, respectively. Rats were orally administered their respective doses daily for 30 days. Results obtained showed that DZN significantly induced thiobarbituric acid reactive substances (TBARS) and decreased the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) and the levels of reduced glutathione (GSH) in rat sera. Aminotransferases (AST, ALT), phosphatases (AlP, AcP) and lactate dehydrogenase (LDH) activities were significantly increased while acetylcholinesterase (AChE) activity was decreased due to DZN administration. Also, DZN treatment caused significant perturbations in lipids profile and serum biochemical parameters. On the other hand, Se alone significantly decreased the levels of TBARS, total lipids, cholesterol, urea and creatinine, while increased the activities of antioxidant enzymes and glutathione content, total protein (TP) and albumin. In addition, Se in combination with DZN partially or totally alleviated its toxic effects on the studied parameters. In conclusion, Se has beneficial effects and could be able to antagonize DZN toxicity.



## Introduction

Pesticides are ubiquitous in the environment and have significant economic, environmental and public health impact. Their usage helps to improve human nutrition through greater availability, longer storage life and lower costs of food. Organophosphate compounds (Ops) are occasionally used indiscriminately in large amounts causing environmental pollution and therefore, are a cause of concern [1,2]. Residual amounts of OP compounds have been detected in the soil, water bodies, vegetables, grains and other foods products [3]. Diazinon (diethoxy-[(2-isopropyl-6-methyl-4-pyrimidinyl) oxy]-thioxophosphorane) is an organophosphorus compound with anticholinesterase mode of action. It is used extensively to control flies, lice, insect pests of ornamental plants and food crops, as well as nematodes and soil insects in lawns and croplands [4]. In addition, oxidant and antioxidant system [1], bio-element levels [2], immune system [5], hematological and biochemical parameters [1] could be affected by OP toxicity. Furthermore, OP insecticides induced toxic effects that probably occur through the generation of reactive oxygen species (ROS), causing damage to various membranous components of the cell [6]. For this reason, treatment with antioxidants and free radical scavengers can decrease the oxidative stress and LPO related to OP-induced toxicity.

Micronutrients are dietary minerals required by the human body in a very small quantity. They probably interact with xenobiotics at several sites like, during absorption and excretion, transport of metals in the body, binding to target proteins, metabolism and sequestration of toxic metals, and oxidative stress [7]. Besides this, they may also serve as required prosthetic groups in active sites or as co-enzymes for indispensable metalloenzymes. Several studies showed that antioxidant nutrients protect cells against deleterious effects of environmental agents [2,8]. Selenium (SE) has received

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considerable attention as an essential micronutrient for both animal and human beings [8]. It has been detected that Se functions in the active site of glutathione peroxidase. It is important in many biochemical and physiological processes including the biosynthesis of coenzyme Q, regulation of ion fluxes across membranes, maintenance of the integrity of keratins, and stimulation of antibody synthesis [7]. The protective effects of Se seem to be primarily associated with its presence in the seleno-enzymes, which are known to protect DNA and other cellular components from oxidative damage [9]. Therefore, the present study was undertaken to evaluate the ameliorating effect of Se on antioxidant status and biochemical changes against diazinon-induced toxicity in serum of male rats.

## Materials and methods

### *Chemicals*

DZN (purity; 99%) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium selenite ($Na_2SeO_3$) used in this study was purchased from Aldrich Chemical Company (Milwaukee, USA). All other reagents used were of analytical reagent grade.

## Animals and care

Twenty-eight male Sprague–Dawely rats (weighing 150–170 g) were obtained from the animal house of the Faculty of Medicine, Alexandria University, Alexandria, Egypt. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups of seven and given food and water ad libitum. The animal room was maintained at 21–24 °C and 40–60% relative humidity with 12-h light–dark cycles, the light cycle coinciding with the day light hours. After 2 weeks of acclimation, the groups were assigned at random to one of the following treatments: group 1 served as control that was given corn oil, while groups 2 and 3 were treated with Se (200 μg/kg BW) and diazinon (10 mg/kg BW), respectively. Group 4 received diazinon (10 mg/kg BW) plus Se (200 μg/kg BW). The diazinon dose used in the present experiment was selected according to the previous study of Ogutcu et al. [10]. Animals were treated daily with the tested compounds by oral gavages for 30 days.

## Serum samples and parameters measured

Blood samples were taken by cardiac puncture and allowed to stand for 30 min at room temperature to clot before being centrifuged at 3000 × *g* for 15 min. Serum was obtained by centrifugation and stored at −60 °C. Serum samples were aliquoted in Eppendorf tubes to use each one for one time. Serum glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. using para-nitrobenzylchloride as a substrate [11]. Superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and Fridovich [12]. The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Catalase (CAT; EC 1.11.1.6) activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of $H_2O_2$, the substrate of the enzyme [13]. The Se-dependent glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC 1.6.4.2) activities were measured by the method described by Hafeman et al. [14]. Glutathione content was measured in serum after reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) using the method of Ellman [15]. Thiobarbituric acid-reactive substances (TBARS) were measured using the method of Ohkawa et al. [16].

Serum alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel [17]. Alkaline phosphatase (AlP; EC 3.1.3.1) activity was measured at 405 nm by the formation of paranitrophenol from para-nitrophenylphosphate as a substrate using the method of Principato et al. [18]. For assaying acid phosphatase (AcP; EC 3.1.3.2) activity, the method of Moss was used [19]. Lactate dehydrogenase (LDH; EC 1.1.1.27) was determined by the method of Cabaud and Wroblewski [20]. Acetylcholinesterase (AChE; EC 3.1.1.7) activity was estimated in serum using acetylthiocholine iodide as a substrate according to the method of Ellman et al. [21]. Serum total cholesterol (TC), triglycerides (TG), total lipids (TL), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and very-low density lipoprotein-cholesterol (VLDL-C) were assayed using commercial reagent kits. Total protein (TP) was determined using the method of Lowry et al. [22]. Folin and Ciocalteus phenol reagent was used to develop the blue color that was measured spectrophotometrically at 750 nm. Bovine serum albumin was used as a standard. Albumin concentrations were determined by the method of Doumas et al. [23]. Globulin concentrations were determined by difference (TP-albumin). Glucose level was measured according to Hyvarinen and Nikkila [24] and concentrations of urea and creatinine were determined by the methods of Patton and Crouch and Henry et al., respectively [25,26]. Total bilirubin was measured using the method of Walters and Gerade [27].

## Statistical analysis

Data were analyzed as a completely randomized design according to Steel and Torrie [28]. Statistical significance of the difference in values of control and treated animals was calculated by ($F$) test with 5% significance level ($P < 0.05$). Data of the present study were statistically analyzed by using Duncan's Multiple Range Test, SAS [29].

## Results

### *Lipid peroxidation and glutathione content*

As shown in Table 1, a significant ($P < 0.05$) increase in TBARS concentration, the indicator of LPO, after the administration of DZN to rats as compared to control while rats treated with DZN + Se showed a significant decrease in TBARS concentration as compared to DZN-treated groups. On the other hand, DZN treated rats showed a significant decrease in glutathione content (GSH). Nevertheless, Se co-treatment to DZN significantly raised GSH content when compared to DZN-treated group. Treatment with Se alone caused significant decrease in serum TBARS, while GSH content was significantly increased.

### *Antioxidant enzymes*

Data concerning serum antioxidant enzyme activities (SOD, CAT, GPx, GR and GST) are presented in Table 1. A significant ($P < 0.05$) reduction in the antioxidant enzymes activity was observed in DZN treated rats as compared to control. However, rats treated with DZN and supplemented with Se, antioxidant enzymes activity showed a significant recovery as compared to DZN treated group. On the other hand, treatment with Se alone caused significant ($P < 0.05$) increase in some of the antioxidant enzyme activities in rat serum.