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Hepatoprotective efficacy of *Nigella sativa* seeds dietary supplementation against lead acetate-induced oxidative damage in rabbit – Purification and characterization of glutathione peroxidase

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ABSTRACT

Lead (Pb) is a toxic ubiquitous environmental pollutant that induces hepatotoxicity in both animals and humans. The ability of *Nigella saliva* seeds (NSS) in ameliorating lead acetate (PbAc)-induced hepatic oxidative damage was investigated using a rabbit model. Forty New Zealand rabbits were given feed and water *ad libitum*. They were allocated randomly into four groups: control; PbAc (5 g/L drinking water); NSS (20 g/kg diet) and NSS+PbAc groups. After two months, liver samples were collected and analyzed for malondialdehyde (MDA), glutathione (GSH), glutathione S-transferase (GST) and glutathione peroxidase (GPx) contents. Purification and characterization of GPx were also evaluated. PbAc exposure significantly ($p < 0.05$) increased MDA (lipid peroxidation biomarker) and reduced the GSH levels and the GST and GPx activities. Concurrently supplemented NSS significantly ($p < 0.05$) decreased MDA levels and restored the GSH, GST, and GPx contents successfully. Electrophoretically, the homogeneous GPx preparation from the liver had a specific activity of 30.44 U/mg protein and a yield of 1.31%. The K_m values for cumene hydroperoxide were 4.76 μM in control, PbAc and NSS+PbAc groups, and 4.09 μM in NSS group. The GPx reaction had a temperature optimum 40 °C, pH optimum 8 and molecular weight 21 kDa. The obtained data indicated the potent efficacy of NSS against PbAc-induced oxidative stress; that was mediated through induction and activation of antioxidants, particularly GPx and scavenging free radicals. Moreover, the purified hepatic GPx is characterized as a selenoprotein (Se-GPx).

1. Introduction

Environmental heavy metals are toxic to various organisms even at very low concentrations, which attributed to their oxidative power and ability to react with other compounds [1]. Air pollution caused by industrial emission, gasoline containing lead (Pb) compound, and food and water contamination are the primary sources of Pb exposure [2,3]. Pb is one of the most utilized metals in industries that induce a broad range of physiological, biochemical, and behavioral dysfunctions [4]. It is a potent systemic toxicant, which causes oxidative damages to the heart, liver, kidneys, reproductive organs, brain, and erythrocytes [5]. Pb can generate reactive oxygen species (ROS) in the body [6] resulting in lipid peroxidation, depletion of cellular antioxidant defense system, and DNA damage [7]. ROS such as superoxide radicals, hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are potentially toxic to the cells and can damage biomolecules [8] that are combated by enzymatic and non-enzymatic antioxidant defenses. Pb induces alterations in enzymatic antioxidant molecules incorporating superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and non-enzymatic antioxidant molecules, such as reduced glutathione (GSH) [9]. In this system, GPx provides detoxification of organic and inorganic peroxides by using GSH [10]. The regeneration of oxidized glutathione (GSSG) is

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achieved by glutathione reductase (GR), which uses NADPH as reduced equivalents [11].

GPx is a selenoprotein, playing an important role in detoxifying the lipids and hydrogen peroxide (H_2O_2), which are formed rapidly during phagocytosis or metabolic processes [12,13]. GPx does not only depend on selenium but also on the availability of GSH for its enzymatic activity [14]. Since it scavenges H_2O_2 in the presence of GSH [15]. GPx has been purified from the cytosol (GPx1), gastrointestinal tract (GPx2), blood plasma (GPx3), phospholipid hydroperoxide (GPx4) [16], epididymis (GPx5), and olfactory system (GPx6) [17]. Therefore, the present study was undertaken to investigate the protective role of dietary supplementation of *N. sativa* seeds against Pb-induced oxidative stress via determination of the hepatic lipid peroxidation and endogenous antioxidants status. Additionally, the current study elucidated the ameliorative effect of *N. sativa* seeds against the inhibitory effect of Pb on the kinetics of the purified hepatic GPx in rabbits.

2. Methods

2.1. Nigella sativa *seeds*

N. sativa seeds (NSS) were purchased from the local market of Herbs and Medicinal Plants, Al-Manshia, Alexandria, Egypt. The identity of NSS was authenticated by Prof. Dr. Azza Shehata and Prof. Dr. Selim Heneidy – Botany Department, Faculty of Science, Alexandria University. A voucher specimen number (El-Far 009/2016) of this plant was maintained in the Department. NSS were thoroughly washed, dried and grinded to a fine powder. After that, it was added and homogeneously mixed with the rabbit's ration at a concentration of 2%.

2.2. Animals and experimentation

All procedures used were approved by the Institutional Animal Care and Use Committee of Alexandria University (Ethical Issue No: VM032/2015). Forty male White New Zealand rabbits (*Oryctolagus cuniculus*) (aged 30 ± 2 days and weighed 900 ± 50 g) were procured from the breeding colony at the Faculty of Agriculture, Alexandria University. The experimental animals were given diet and water *ad libitum*. The rabbits were housed in metal box cages at controlled temperature (28 ± 2 °C) and relative humidity 60±10% with a 12-h light/dark cycle. After two weeks of acclimatization, animals were allocated into four groups (10 rabbits/each). The control group was fed freely on basal diet and water. Lead acetate [PbAc, $Pb(CH_3COO)_2.3H_2O$] group was fed on basal diet and received water containing PbAc (5 g/L) [18]. NSS group was fed on a basal diet containing NSS (20 g/kg diet), while water offered freely. PbAc+NSS group was fed on a basal diet containing NSS and received water containing PbAc. All the treatments were daily applied for two consecutive months. The basal diet is consisting of alfa-alfa hay (33.0%), yellow corn (20.0%), barely (19.3), soya bean meal (14.0%), wheat bran (8.0%), molasses (5.0%), salt (0.5%) and vitamin (0.2%). It was formulated to meet all nutritional requirements of growing rabbits for minerals, vitamins, protein, essential amino acids and metabolizable energy [19,20].

2.3. Preparation of liver tissue homogenate

Twenty-four hours after the end of the experimental period, the rabbits of control and experimental groups ($n = 10$) were sacrificed under anesthesia with an intravenous injection of sodium pentobarbital (30 mg/kg bw), and then liver samples were immediately dissected and soaked in ice-cold saline 0.9%. They were homogenized using a motor-driven Teflon and glass Potter-Elvehjem homogenizer in 0.1 M Tris-HCl buffer of pH 7.4 containing 5 mM β-mercaptoethanol (1:4 *w*/*v*). The homogenates were centrifuged at $105{,}000 \times g$ for 60 min at 4 °C; the supernatants were divided into aliquots then stored at −20 °C for further evaluation of oxidative stress and purification of GPx enzyme.

2.4. Determination of oxidative stress parameters

The frozen aliquots of liver homogenates were utilized for the colorimetric assessment of MDA and GSH contents, as well the GST and GPx enzymes activities.

2.4.1. Determination of lipid peroxidation

Malondialdehyde (MDA) is the main aldehyde by-product of lipid peroxidation in biological systems. It was analyzed after the incubation of supernatants with thiobarbituric acid at 95 °C for 30 min (pH 3.6) to form thiobarbituric acid-reactive substances (TBARS), a pink colored compound. MDA levels were measured at 532 nm and expressed as nmol MDA/mg proteins [21].

2.4.2. Determination of reduced glutathione (GSH) levels

Reduced glutathione assay was based on the reductive cleavage of DTNB [5,5′-dithiobis (2-nitrobenzoic acid)] by compounds containing sulfhydryl groups and development of a yellow color [22]. The quantity of reduced chromogen is directly proportional to the GSH content. The absorbance was recorded at 412 nm and expressed as μmol GSH/mg proteins.

2.4.3. Determination of the glutathione S-transferase (GST) activity

The activity of GST was assayed according to the method of Vessey and Boyer [23]. This assay was based on monitoring the rate of enzyme–catalyzed conjugation of the CDNB [1-chloro-2,4-dinitrobenzene] with GSH. GST activity was measured as the increase in absorbance at 340 nm and represented as l mol CDNB/min/mg protein (A€=9.6/mM/cm).

2.4.4. Determination of the glutathione peroxidase (GPx) activity

GPx activity was evaluated according to the method of Chiu, Stults and Tappel [24]. The assay is based on measuring the oxidized glutathione (GSSG), which is produced by the reduction of organic peroxide in a reaction mixture contained GSH, GR, NADPH, and Tris–HCl. GPx activity was measured as the increase in absorbance at 340 nm and represented as GPx units/mg of protein.

2.4.5. Determination of tissue Protein

Protein concentrations in the tissue homogenates were determined using bovine serum albumin as the standard according to the method of Bradford [25].

2.5. Purification and characterization of glutathione peroxidase (GPx) enzyme

To investigate the effect of PbAc on GPx kinetics, and to monitor the degree of protection of NSS against PbAc, GPx was purified from liver tissues following the modified method of Duan, Komura, Fiszer-Szafarz, Szafarz and Yagi [26].

2.5.1. Ammonium sulfate salting out

Ammonium sulfate powder was added slowly to the supernatants of liver homogenates until 90% saturation. After overnight incubation, centrifugation at $15{,}000 \times g$ for 20 min at 4 °C was done, and the precipitate containing the GPx enzyme was collected and dissolved in 130 ml of 10 mM Tris buffer (pH 8.0) containing 5 mM β-mercaptoethanol (Buffer A) and dialyzed against the same buffer overnight.