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Original Research

Barley phenolic compounds impedes oxidative stress in lead acetate intoxicated rabbits

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Key Words
Barley; Glutathione; Glutathione S-transferase; Glutathione peroxidase; Malondialdehyde; Rabbits

Abstract
This study was conducted to explore the effects of lead acetate and barley phenolic compounds, alone or in combination, on lipid peroxidation marker and some antioxidant enzymes using a rabbit model. Rabbits were provided with drinking water containing lead acetate (500 ppm) and/or gastro-gavaged with barley phenolic compounds (250 mg/kg/day) for 30 days. Following overnight fasting, the animals were sacrificed, and the levels of glutathione and malondialdehyde and the activities of glutathione S-transferase and glutathione peroxidase in brain, liver and kidney tissues were measured spectrophotometrically. Treatment with lead acetate significantly decreased the activities of glutathione S-transferase, glutathione peroxidase and the level of reduced glutathione, and it significantly increased the level of lipid peroxidation marker. Conversely, treatment with barley phenolic compounds, whether alone or in combination with lead acetate, significantly increased the activity of antioxidant enzymes and the level of reduced glutathione, and significantly decreased the level of lipid peroxidation marker. The use of barley phenolic compounds were successive to ameliorate the toxic effects of lead acetate, as reflected in the decreased levels of lipid peroxidation marker and the increased antioxidant activity.

INTRODUCTION

Oxidative stress results when the production of reactive oxygen species exceeds the antioxidant capability of cells or systems. The accumulation of oxidative damage has been implicated in both acute and chronic cell injury and may contribute to the onset of cancer [1], and liver and heart diseases [2]. Antioxidants are compounds that delay the initiation or slow down the rate of substrate oxidation.

The uses of plant-based antioxidant compounds in foods and preventive medicine are very interesting because of their potential health benefits [3]. It is well accepted that plants are the rich sources of antioxidants. Among plants, cereals and legumes are prominent as sources of antioxidants because they contain a wide array of phenolic compounds [4, 5]. Phenolic acids occur in the cereal grains primarily as conjugates with sugars, fatty acids, or proteins and act as effective natural antioxidants [6]. Plant phenolics have multiple biological functions, including antioxidant, anti-inflammatory, anti-cancer and anti-microbial activities [7].

Barley (*Hordeum vulgare* L), a member of the grass family Poaceae, is grown around the world as a principal cereal crop [8]. Barley grains have been used for several food purposes, both as whole grains and in the form of value-added products. They contain a wide array of phytochemicals, primarily phenolic compounds that include flavonols, phenolic acids, and procyanidins. The major phenolic components of barley are ferulic, caffeic, and vanillic acids [9]. Barley may exert an inhibitory effect on hydrogen peroxide (H_2O_2) by blocking H_2O_2-induced oxidative damage to DNA, cell death and apoptosis [10]. Barley also has hepatoprotective and hypolipidemic effects [11]. Barley β-glucan improves milk chemistry [12].

Heavy metals are among the most widespread potential chemical contaminants in the environment and are

transferable to man and animals through dietary and other routes [13]. The heavy metal lead causes a variety of toxic effects when ingested or inhaled [14]. Animals and poultry can be affected by lead-contaminated water resulting from industrial activity. The nervous system is the primary target of the toxic effects of lead exposure [15]. In poultry, lead poisoning causes anemia, abnormal hepatorenal function, muscular pain, and neuropathy of both central and peripheral nervous systems [16].

Studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and reducing the antioxidant defense system of cells by depleting glutathione, inhibiting sulfhydryl dependent enzymes, interfering with metals that are essential for antioxidant enzyme activities, and/or increasing the susceptibility of cells to oxidative attack by altering the integrity and fatty acid composition of the membrane [17]. An impaired oxidant/antioxidant balance may be partially responsible for the toxic effects of lead [18]. This study assessed the adverse effects of lead acetate on some antioxidant enzymes *in vivo* and the possible use of barley as a natural protection remedy.

MATERIALS AND METHODS

This study was carried out in Department of Biochemistry, Faculty of Veterinary Medicine, Damanhur University, Egypt, after approval of Dean of Faculty and of Head of Biochemistry department.

Chemicals

5,5-dithio-bis-2-nitrobenzoic acid (DTNB), disodium salt of ethylenediaminetetraacetic acid (EDTA), cumene hydroperoxide and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma (St. Louis, MO, USA). Thiobarbituric acid (TBA) and reduced glutathione (GSH) were obtained from Fluka (Milwaukee, WI, USA). Trichloroacetic acid (TCA) and β-mercapto-ethanol were obtained from Merck (Whitehouse Station, NJ, USA). Lead acetate was kindly provided by the National Research Center, Dokki, Egypt and was used at a concentration of 500 ppm.

Plant authentication

Barley (*Hordeum vulgare* L) grain was obtained from local market and was authenticated in Department of Nutrition, Faculty of Agriculture, Damanhur University, to determine its composition.

Plant extraction

Barley grains were ground in a Moulinex coffee mill. Phenolic compounds were extracted from the ground material using 80% (v/v) methanol at the solids-to-solvent ratio of 1:10 (w/v) at 50ºC for 30 min [19]. The extraction was carried out in dark-colored flasks in a shaking water bath. The extraction was repeated twice more, the supernatants were combined and methanol was evaporated under vacuum at 40ºC using a rotary evaporator. Residual water was removed from the extract by lyophilisation. The crude extract was stored at –20ºC until used.

Column chromatography

An 800-mg dried sample of the barley crude extract was dissolved in 8 ml of methanol, applied to the top of the chromatographic column (ID/length; 20 x 800 mm), packed with Sephadex LH-20 (bead size: 25-100 μ), and then eluted from the column with methanol as the mobile phase. Fractions (8 ml) were collected using a LKB Bromma 2112 RediRac fraction collector and their absorbances were measured at 280 nm. Eluates were then pooled into major fractions, the solvent was removed under vacuum in a rotary evaporator, and the products were weighed.

Total phenolics

The contents of total phenolic compounds in the barley fractions were estimated using the Folin-Ciocalteu reagent. (+)-Catechin was used as the standard in this work. The most active fractions were collected, concentrated and resuspended in distilled water and administered orally at dose rate of 250 mg/kg/day.

Animals

Forty rabbits (3.5 months old, weighing 1,000-1,200 g) were divided into four equal groups. The first group was the control group. The drinking water of the second group was supplemented with 500 ppm lead acetate [14]. The third group was supplemented with barley extract at dose rate of 250 mg/kg daily by stomach tube. The fourth group received both drinking water containing 500 ppm lead acetate and barley extract at dose rate of 250 mg/kg daily by stomach tube. The rabbits were acclimatized for three weeks before the start of the experiment and monitored for normal growth and behavior. The experimental period lasted for 30 days.

Samples

At the end of the experiment, the rabbits were fasted overnight prior to being sacrificed. The brain, liver and kidneys were removed and washed with ice-cold Tris-HCl buffer (0.05 M, pH 7.4) containing 0.25 M sucrose. The tissues were blotted, dried, weighed and then homogenized in the ice-cold buffer with twelve strokes in a tight-fitting Potter-Elvehjem homogenizer. Malondialdehyde (MDA) was used as an indicator of lipid peroxidation and was measured spectrophotometrically following reaction with thiobarbituric acid [20]. The activity of glutathione S-

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transferase (GST) was determined spectrophotometrically at room temperature by monitoring the rate of GSH conjugation with CDNB [21]. The activity of glutathione peroxidase (GPx) was determined chemically using cumene hydroperoxide as substrate [22]. The level of GSH was assayed by spectrophotometric monitoring of the reductive cleavage of DTNB by a free sulfhydryl group at 412 nm [23]. Blood lead level was assayed by atomic absorption.

Statistics

The results are expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant.

RESULTS

The administration of lead acetate significantly ($P < 0.05$) increased lipid peroxidation, as shown by increased levels of MDA levels in brain, liver and kidney tissue (Table 1). In contrast, supplementation with barley produced a significant ($P < 0.05$) decrease in the level of MDA relative to both the control and lead acetate groups.

Administration of lead acetate significantly decreased ($P < 0.05$) the activity of GPx in brain, liver and kidney tissue (Table 2). Co-treatment with barley, however, significantly increased ($P < 0.05$) the activity of GPx relative to the lead acetate group.

Lead acetate significantly decreased ($P < 0.05$) the activity of GST in brain, liver and kidney tissue (Table 3). Treatment with barley and lead acetate together significantly ($P < 0.05$) increased the activity of GST relative to the lead acetate group.

The administration of lead acetate significantly decreased ($P < 0.05$) the level of GSH in brain, liver and kidney tissue (Table 4). Supplementation with barley significantly ($P < 0.05$) increased the level of GSH relative to the lead acetate group.

Lead acetate significantly increased ($P < 0.05$) blood lead level (Table 5). While barley phenolics either alone or in combination with lead acetate significantly ($P < 0.05$) decreased blood lead level in compare with lead acetate group.

Table 1. Effect of lead acetate and barley phenolics on lipid peroxidation

Groups	MDA (nmol/g wet tissue)		
	Brain	Liver	Kidney
Group I (control)	102.06 ± 1.71^{c}	127.1 ± 1.08^{b}	122.93 ± 1.45^{c}
Group II (lead acetate)	176.8 ± 1.32^{a}	188.94 ± 1.93^{a}	171.66 ± 1.05^{a}
Group III (barley phenolics)	87.66 ± 1.17^{d}	109.3 ± 1.89^{c}	107.7 ± 1.75^{d}
Group IV (lead acetate + barley phenolics)	141.96 ± 1.76^{b}	129.7 ± 1.39^{b}	152.92 ± 1.35^{b}

Different superscript letters indicate significant differences ($P < 0.05$); LP, lipid peroxidation; MDA, malondialdehyde.

Table 2. Effect of lead acetate and barley phenolics on glutathione peroxidase activity

Groups	GPx activity (IU/g wet tissue)		
	Brain	Liver	Kidney
Group I (control)	25.64 ± 0.53^{b}	29.48 ± 1.59^{a}	23.03 ± 0.47^{ab}
Group II (lead acetate)	16.25 ± 0.17^{d}	8.01 ± 1.45^{c}	7.98 ± 0.35^{c}
Group III (barley phenolics)	33.73 ± 0.39^{a}	30.03 ± 1.5^{a}	27.67 ± 0.71^{a}
Group IV (lead acetate + barley phenolics)	20.79 ± 0.27^{c}	15.16 ± 1.85^{b}	18.75 ± 0.32^{b}

Different superscript letters indicate significant differences ($P < 0.05$); IU, international units; GPx, glutathione peroxidase.

Table 3. Effect of lead acetate and barley phenolics on glutathione-S-transferase activity

Groups	GST activity (mol CDNB/min/g wet tissue)		
	Brain	Liver	Kidney
Group I (control)	318.3 ± 108.5^{c}	735.3 ± 57.09^{b}	448.5 ± 0.95^{b}
Group II (lead acetate)	161.2 ± 94.62^{d}	203.4 ± 37.47^{d}	163.8 ± 0.43^{d}
Group III (barley phenolics)	489.6 ± 128.7^{a}	829.6 ± 52.09^{a}	669.6 ± 0.59^{a}
Group IV (lead acetate + barley phenolics)	382.6 ± 99.62^{b}	526.4 ± 39.57^{c}	327.4 ± 0.41^{c}

Different superscript letters indicate significant differences ($P < 0.05$); GST, glutathione S-transferase; CDNB, 1-chloro-2, 4-dinitrobenzene.

Table 4. Effect of lead acetate and barley phenolics on glutathione levels

Groups	GSH (μmol/g wet tissue)		
	Brain	Liver	Kidney
Group I (control)	27.49 ± 1.66^{b}	63.02 ± 1.37^{c}	61.95 ± 1.74^{b}
Group II (lead acetate)	18.85 ± 0.47^{c}	42.39 ± 1.04^{d}	49.03 ± 1.91^{c}
Group III (barley phenolics)	33.51 ± 1.6^{a}	131.48 ± 1.73^{a}	93.39 ± 2.27^{a}
Group IV (lead acetate+ barley phenolics)	26.97 ± 1.47^{b}	88.61 ± 1.34^{b}	63.11 ± 1.61^{b}

Different superscript letters indicate significant differences ($P < 0.05$); GSH, glutathione.

Table 5. Effect of lead acetate and barley phenolics on blood lead level (μg/dl)

Groups	Blood lead level (μg/dl)
Group I (control)	12.45 ± 3.16^{c}
Group II (lead acetate)	79.98 ± 4.67^{a}
Group III (barley phenolics)	10.87 ± 1.2^{c}
Group IV (lead + barley)	48.76 ± 2.76^{b}

Different superscript letters indicate significant differences ($P < 0.05$).

DISCUSSION

As shown in Table 1, the administration of lead acetate in drinking water significantly increased the level of MDA in rabbit tissues. MDA is one of lipid peroxidation markers and the increased level indicates oxidative stress induced by lead toxicity [24, 25]. It has previously been shown that lead induce lipid peroxidation in neonatal rat brain [26]. Lead also induced lipid peroxidation in serum, liver and different parts of mature rat brain [14, 27]. Exposure to lead caused increased plasma lipid peroxidation in goats [28]. Lead may induce lipid peroxidation by at least two different mechanisms: *(i)* inhibition of delta amino levulinic acid dehydratase (δ-ALAD) by lead causes accumulation of delta amino levulinic acid (δ-ALA), which can be rapidly oxidized to generate free radicals such as superoxide ion ($O_2\bullet^-$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2); and *(ii)* lead can directly stimulate the initiation of membrane lipid peroxidation by ferrous (Fe^{2+}) ion [29].

Enzymatic antioxidant defense systems naturally protect cells against lipid peroxidation. Glutathione protects cells from oxidative damages [30]. Treatment with lead acetate caused significant reductions in the activities of GPx and GST, and GSH levels in rabbit brain, liver and kidney tissue (Tables 2-4). These results suggest that the level of GPx was depleted by countering the increased amount of free radicals induced by lead acetate in these tissues. These data are consistent with previous lead toxicity studies that demonstrated a significant decrease in the serum total antioxidant level (TAO) in chicken [31] and rats [23, 24, 32]. Lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and reducing the antioxidant defense system of cells [17]. These authors demonstrated that lead decreased the activity of antioxidant enzymes by depleting GSH, inhibiting sulfhydryl-dependent enzymes, interfering with metals that are essential for the activity of antioxidant enzyme activities, and/or increasing the susceptibility of cells to oxidative attack by altering the integrity and fatty acid composition of the membrane. Rats and goats treated with lead exhibited decreased blood levels of CAT, GPx, SOD and GR [14, 28]. Conversely, Kiran *et al* [27] showed that exposure to lead resulted in significant increases in the activity of the antioxidant enzymes catalase and superoxide dismutase. The differences in the results of this study, when compared with previous observations, may be attributed to differences in dose, duration of exposure and model species.

Whether alone or in combination with lead acetate, dietary barley significantly decreased the lipid peroxidation of and significantly increased the antioxidant status of the tested tissues (Tables 1-4). These observations are consistent with previous reports that the values of MDA and total antioxidants in chicken that had been treated with lead acetate returned to control levels after barley was added to the diet [10, 31]. Whole grains contain many antioxidants, including vitamins (*e.g.*, vitamin E), trace minerals (*e.g.*, selenium, copper, zinc and manganese) and non-nutrients such as phenolic acids, lignans and phytoestrogens, and anti-nutrients such as phytic acid [33]. Barley contains high concentrations of phytic acid, a known antioxidant that chelates with various metals and suppresses damaging iron-catalyzed redox reactions, subsequently reducing levels of lipid peroxidation [34]. Moreover, vitamin E is naturally present in barley, it is an intracellular antioxidant that protects polyunsaturated fatty acids in cell membranes from oxidative damage. Another possible antioxidant mechanism for vitamin E arises from its ability to stabilize reduced selenium [35].

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The phenolic compounds in barley participate in a potentially anti-carcinogenic mechanism that involves the induction of detoxification systems, specifically the Phase II conjugation reactions (GST) [36]. Selenium is also naturally present in barley, but is removed in the refining process. The selenium content of barley food products is proportional to the selenium content of the soil in which the grain is grown. Selenium functions as a cofactor for glutathione peroxidase, an enzyme that protects against oxidative tissue damage. It also suppresses cell proliferation at high concentrations.

In conclusion, barley can protect to some extend against lead acetate induced oxidative stress reflected in significant decreased lipid peroxidation and significant increased antioxidant enzymes. This may be attributed to its contents of antioxidants compounds.

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DECLARATION OF INTEREST

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