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Protective effects of date extract against toxic effects of lead acetate in rats.

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Abstract

Lead (Pb) is a common industrial and environmental pollutant. Prolonged exposure of a sublethal dose to this toxicant is associated with oxidative stressand considered to be a risk factor for kidney, liver in addition to many disorders. This study was carried out to investigate the most toxic effects of lead with trial to diminish this toxicity by supplementation of date extract. Both the enzymatic {superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GSH-T) and catalase (CAT)} and non-enzymatic (gutathione, GSH) antioxidants had been studied, among mature male albino rats which have been received lead acetate in drinking water for 3 months. it is widely accepted that even small quantities of Pb are harmful to rats which implicated in a broad range of physiological conditions.the study proved that the use of date extract, through its antioxidant protective effect and immune potentiating properties, can reduce Pb-induced hepatic damage and toxicity

Introduction

Lead is ubiquitous, and the most common environmental pollutant naturally present in the earth's crust in small concentrations, For centuries, it has been mined and disseminated throughout the environment from where it has gradually become incorporated into the structural tissue of plants, animals and humans (**Pracheta *et al.*, 2009**). There are two sources of exposure, i.e. exposure to lead based, paint and exposure to lead related industries, which are either inevitable or reducible at a rate so slow that is still a threat to public health (**Martin *et al.*, 2001**).

Lead pollution can also cause irreversible encephalopathy, seizure, coma and even death. Fatigue, memory loss, high blood pressure, nephropathy, gastrointestinal disturbances, weight loss and immuno-suppression are other common toxic effects of lead exposure in animals. Prenatal exposure to metal may also cause birth defects, miscarriage and underdeveloped babies (**Ehle and Mckee, 1990 and Pracheta *et al.*, 2009**).

The date fruit is listed in folk remedies for the treatment of various infectious diseases and cancer (**Duke, 1992**). The aqueous extracts of dates have potent antioxidant and antimutagenic activity (**Allaith, 2007; Biglari *et al.*, 2008 and Saafi *et al.*, 2009**). This study was carried out to investigate the most toxic effects of lead with trial to diminish this toxicity by supplementation of date extract

Materials and Methods

This study was carried out at the Unit for Laboratory Animals at Hygiene and Management Department, Faculty of Veterinary Medicine, Cairo University.

3.1 Animals:

The study was conducted on Immature Wistar male rats (N=120) weighing approximately 120-140 g were obtained from the Unit for Laboratory Animals at Faculty of Veterinary

Medicine, Cairo University. The temperature in the laboratory animal house unit ranged from 25-30°C. The relative humidity ranged from 50-70%. Natural system of lighting was used throughout the study .Cages were cleaned twice weekly in summer and once per week in winter. Animals care as well as experimental protocols were in compliance with guidelines of ethical standards released by Cairo University policy on animal care and use. All efforts were made to minimize the numbers of animals and their suffering in this study through following the Guidelines on Laboratory Animal Care and Use.

3.2 **Preparation of date palm extracts:**

Fresh ripened Wahat date variety collected from the Wahat was extracted two times with distilled water (1/10, w/v) by grinding with a mortar and pestle. It was centrifuged at 41C for 20 min at 4000 g and the supernatant was collected.

3.3 **Experimental design**

Immature male rats (120) were divided at random into 4 groups of 30 animals, each as follows:

Group 1: Control group: animals received a daily distilled water as drinking water for 12 weeks.

Group 2: High lead acetate group: animals received a daily lead acetate at a concentration of 0.5 mg / liter dissolved in distilled water and administered to the rats in drinking water for 12 weeks.

Group 3: Low lead acetate group: animals received a daily lead acetate at a concentration of 0.2 mg / liter dissolved in distilled water and administered to the rats in drinking water for 12 weeks.

Group 4: low lead acetate with date extract: animals received a daily oral of lead acetate at a concentration of 0.2 mg / liter dissolved in distilled water with date extract (4 ml / kg) for 12 weeks .

3.4 Determination of oxidative stress and lipid peroxidation:

Tissues (liver, spleen, brain and kidney) and blood were homogenized in ice-cold 1.15% KCl to make 10% (W/V) homogenate with Glas-Col motor driven homogenizer (USA) and the homogenate was used for determination of malondialdehyde (MDA), reduced glutathione (GSH) levels as well as superoxide dismutase (SOD), catalase (CAT), Glutathione transferase (GST),and Glutathione Reductase (GSR) activities as recommended by (**Buege and Aust,(1978), Burtes and Ashwood (1999) and Kakkar *et al.* (1984)**; respectively.

3.5 **Determination of lipid peroxidation (MDA) concentration:**

MDA concentration was assayed using the commercial MDA Assay Kit was purchased from (Bio Diagnostic Co., Egypt).The MDA adduct can be easily quantified colorimetrically at $\lambda = 532$ nm .

3.6 Determination of reduced glutathione (GSH) (Burtis and Ashwood,1999):

GSH concentration was assayed using the commercial GSH Assay Kit was purchased from (Bio Diagnostic Co., Egypt).

3.7 Determination of superoxide dismutase (SOD) Activity:

SOD concentration was assayed using the commercial SOD Assay Kit was purchased from (Bio Diagnostic Co., Egypt).

3.8 Determination of catalase (CAT) Activity:

CAT activity was assayed using the commercial CAT Assay Kit was purchased from (Bio Diagnostic Co., Egypt).

3.9 Determination of glutathione-S-transferase activity:

GST activity was assayed using the GST commercial Colorimetric Activity Assay Kit was purchased from (Bio Diagnostic Co., Egypt) anddetected by spectrophotometry at 340 nm. One unit of GST activity is defined as the amount of enzyme producing 1 /mol of GS-DNB conjugate/min under the conditions of the assay (**Tounget al., 1990**).

3.10 Determination of Glutathione Reductase Activity:

GR concentration was assayed using the commercial GR Assay Kit was

purchased from (Bio Diagnostic Co., Egypt).

Results and Discussion:

Lead is a metal with many recognized adverse health side effects (**Sui *et al.*, 2015**). It is a ubiquitous environmental and industrial pollutant that induces a broad range of toxic manifestations within biological systems.Exposure to lead induces over-production of reactive oxygen species and depletes the cellular antioxidant capacity. An imbalance of pro-oxidant/antioxidant ratio in tissue and cellular components is known to cause damage to membranes, DNA, or proteins, and finally destroy the tissues or systems (**Hsu and Guo, 2002**).

Lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS), reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzymes or antioxidant enzymes activities or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition (**Sharma *et al.*, 2011**).

In the present study, both the enzymatic SOD, GR, GPx, GSHT and CAT and non-enzymatic (glutathione GSH) antioxidants had been studied.

Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. Lipid peroxidation forms Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as natural bi-products. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. Pb is known to alter the activity of lipid metabolizing enzymes in liver (**Kojima *et al.*, 2005**), which can limit the biosynthesis of bile acids. Bile acid plays important role in elimination of cholesterol from the body (**Mudipalli, 2007; Newairy and Abdou, 2009**) in addition to increasing lipid peroxidation (**Kamalakkanan and Prince, 2004**).

Pb is known to produce oxidative damage by elevating peroxidation of membrane lipids. Generation of peroxyl radicals after Pb-intoxication stimulates lipid peroxidation via a cyclisation reaction to endoperoxides (**Marnett, 1999 and Sharma *et al.*, 2010**).

Lead may disturb the antioxidant barrier via inhibition of the functional SH groups present also in free radical-scavenging enzymes such asGR, GPx,GST ,SOD, CAT and d-aminolevulinic acid dehydratase (D-ALAD) (**Moniuszko-Jakoniuk *et al.*, 2007; Olaleye *et al.*, 2007**). These enzymes are believed to be the major antioxidant agents in the mammalian body that protect against ROS toxicity (**Ashry *et al.*, 2010**).

The observed increase in the MDA was accompanied by a 70% decrease in GSH levels in the PbAc-group, which may be due to its utilization either in lead detoxification or in scavenging lead-generated free radicals (**Othman and El-Missiry, 1998 and Olaleye *et al.*, 2007**). Under oxidative stress, the GSH-related enzymes merely catalyze reactions to detoxify peroxides in the water phase by reacting them with GSH (**Moniuszko-Jakoniuk *et al.*, 2007**).

Thus, the enhanced concentration of MDA and severe depletion in GSH activity suggests that the increased peroxidation is a consequence of depleted GSH stores and diminished GSH-related enzymes, which are otherwise capable of moderating the degree of lipid peroxidation (**Ashry *et al.*, 2010**). It is hypothesized that increased lipid peroxidation as a result of reduced endogenous antioxidant capacity may be the initial event in the mechanism of Pb-induced hepatic damage (**Ashry *et al.*, 2010**).

In this study, Pb intoxication caused significant enhancement of thio barbituric acid reactive substances (TBARSs) levels in blood and organs (liver, spleen, brain and kidney). Date extract significantly lowered TBARSs levels in blood and other organs in a dose dependant manner. The protective effect may be due to the radical scavenging activity of phenolics present in the extract. Ubiquinones (co-enzymes Q)

function as important cellular electron carriers **(Ernster and Dallner, 1995)**

The result of SOD and CAT activity suggest that date extract contains a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of free radicals. This action could involve mechanisms related to scavenging activity.

We selected an aqueous extract because most of the antioxidant components in dates are extracted in water **(Vayalil, 2002 and Al-Farsi *et al.*, 2005b)**. During the experience, the aqueous date fruit extract (Wahat date) was daily prepared and administrated to rats.

The aqueous extracts of dates have potent antioxidant and anti-mutagenic activity, the antioxidant activity is attributed to its content of the wide range of phenolic compounds including p-coumaric, ferulic, and sinapicacids, flavonoids and procyanidins **(Farsi *et al.*, 2005; Mansouri *et al.*, 2005 and Hong *et al.*, 2006)** and also to the presence of vitamin C **(Allaith, 2007 and Mrabet *et al.*, 2008)**. Flavonoids can act in the initiation stage of peroxidation interfering with the metabolism oxidative agent either by scavenging the free radicals or by impairing the microsomal enzymatic system needed for this metabolism **(Singh and Handa, 1995)**. It is clear that date extract showed an antioxidant protective effect

Table (1): Mean ± SD of oxidative stress and lipid peroxidation parameters among rats which received lead acetate.

	Control group	High lead acetate treated group	Low lead acetate treated group	Date extract with low lead acetate treated group
G.S.H	9.80 ± 1.92 a	4.00 ± 2.00 c	6.80 ± 2.39 b	8.40 ± 1.52 ab
G.R	12.40 ± 2.07 c	45.80 ± 8.04 a	33.60 ± 4.72 b	18.00 ± 5.43 c
G.Px	16.00 ± 4.06 c	46.40 ± 8.08 a	37.20 ± 5.17 b	23.00 ± 5.24 c
MDA	14.00 ± 4.06 c	17.60 ± 3.91 bc	21.40 ± 4.39 b	29.80 ± 4.32 a
CAT	56.00 ± 2.65 c	65.40 ± 5.03 b	78.00 ± 8.37 a	48.80 ± 5.63 c
SOD	610.40 ± 17.84 c	698.80 ± 16.75 b	839.60 ± 32.54 a	579.60 ± 6.43 d

Means with different letters (a, b, c, d) within the same row are significantly different at P value ≤ 0.05
G.S.H: Glutathione G.S.H.R: Glutathione Reductase G.S.H.Px: Glutathione peroxidase MDA: Malondialdehyde concentration CAT: Catalase Activity SOD: Superoxide Dismutase Activity.

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الملخص العربي

الرصاص هو من الملوثات الصناعية والبيلية المشتركةويرتبط التعرض لفترات طويلة لجرعات اقل من المميتة ذو تاثير علي الكلي والكبد ويؤدي إلي العديد من الاضطرابات. وقد أجريت هذه الدراسة للتحقيق في الآثارالأكثر سمية للرصاص مع محاولة تقليل هذه السمية عن طريق إضافة مستخلص ثمار البلح.

ويتم في هذه الدراسة دراسة تاثير الرصاص علي بعض النزيمات الأكسدة في الكبد والكلي بين ذكور الفئران الناضجة حيث تتلقي ذكور الفئران الناضجة خلات الرصاص في مياه الشرب لمدة 3 شهور. وفي الختام اتضح أنه حتي كميات صغيرة من الرصاص ضارة للفئران.واتضح أيضا تاثير مستخلص البلح المضاد للأكسدة في الظروف الفسيولوجية من خلال تاثير وقائي وبالتالي يؤدي الي رفع الخصائص المناعية ويؤدي إلي تقليل تاثير الرصاص علي الكبد والكلي .