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Spirulina or dandelion-enriched diet of mothers alleviates lead-induced damages in brain and cerebellum of newborn rats

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ABSTRACT

This study was aimed at evaluating the toxic effects of a prenatal exposure to lead acetate on brain tissues of newborn rats, and potent protective effects of spirulina *(Arthropira platensis)* or dandelion *(Taraxacum officinalis)* added to rat diet. Female rats were given a normal diet (control) or a diet enriched with spirulina or dandelion. Additionally, lead acetate was administered to one half of these rats through drinking water from the 5th day of gestation, to day 14 postpartum. Lead toxicity was assessed by measuring blood lead levels, brain weight, tissue damage, as well as protein content, lipid peroxidation and activities of antioxidant enzymes in brain tissues of neonates. Lead poisoning of mothers caused lead deposition in the brain and cerebellum of newborns and cerebellum tissue damages. Moreover, a significant decrease in weight and protein content of these tissues was found. Oxidative stress and changes in antioxidant enzyme activities in brain tissues were also recorded. Conversely, no such damages or biochemical changes were found in neonates from plant fed lead-poisoned mothers. These results strongly suggest that beneficial effects of spirulina- or dandelion-added diet on lead-intoxicated rats proceeded through the reduction of the lead-induced oxidative stress and related damages.

1. Introduction

Acute or chronic intoxication of animals and humans by lead is well documented. Lead (Pb) is one of the most widely used metals in industries and in many countries exposure to Pb continues to be a widespread problem. Animals and humans may get exposed to Pb due to food or water contamination, and to air pollution caused by industrial emission and combustion of lead-containing gasoline (Pande and Flora, 2002; Mudipalli, 2007). The toxicity of lead has been known for centuries, and symptoms caused by Pb in the hematopoietic, gastrointestinal, urinary, cardiovascular, and nervous systems are well described (Goyer, 1993; Aggarwal et al., 2007).

Rat exposure to lead was found to cause spatial learning deficits (Kuhlmann et al., 1997), in relation with the accumulation of lead in the hippocampus, the critical brain region in learning and memory (Kumar et al., 2009). Many of the adverse effects of lead exposure have been attributed to the propensity of lead to induce the production of reactive oxygen species (ROS), DNA damage, and inactivation of anti-oxidant enzymes (Gurer-Orhan et al., 2004; Kumar et al., 2009). Indeed, in both young and adult rats, lead treatment was found to cause alteration in expression levels of various anti-oxidant enzymes in the brain, including superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPX), and guanylate cyclase (Farmand et al., 2005; Bennet et al., 2007; Bokara et al., 2008).

One alternative to prevent lead-induced oxidative damages in animals is to provide a diet enriched with antioxidants. With that respect, antioxidant-rich plants could be of valuable use. Among them, spirulina (*Arthrospira platensis)* has been reported to possess high levels of antioxidant phycocyanins (Bhat and Madyastha, 2001). This microalgae had several medicinal (Roy et al., 2007; Sharma et al., 2007) and nutritional properties, the latter deriving from its high content in proteins and natural biochelated vitamins (Kapoor and Mehta, 1993; Simpore et al., 2006). Besides, dandelion

Abbreviations: CAT, catalase; GPX, glutathione peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TBS, tris-buffer saline.

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(*Taraxacum officinale* L., Asteraceae) is an edible perennial traditionally used as an herbal medicine due to its antidiabetic, choleretic, antirhematic, and diuretic properties (Schütz et al., 2006). Recent studies have provided evidence that it may also reduce inflammation and tumour risks (Yanghee et al., 2010).

The aim of this study was to investigate the effects of diets enriched with spirulina and dandelion in rat neonates after oxidative stress induced by metal (lead) intoxication.

2. Materials and methods

2.1. Animals

Wistar rats weighing 170 to 180 g were obtained from the "Central Pharmacy of Tunis" (SIPHAT). They were kept in cages in a breeding farm at a temperature of 21 ± 1 °C with alternating periods of 14/10 h of darkness/illumination, with a relative humidity around 40%. All animals had free access to drinking water. The basic food was 15% protein industrial pellets provided by the Industrial Society of Concentrate (SICO, Sfax, Tunisia).

The experimental procedures were carried out according to the general guidelines on the use of living animals in scientific investigations (Council of European Communities, 1986) and approved by the Ethical Committee of the Faculty of Science of Sfax.

2.2. Plants

Spirulina (*A. platensis*) variety Lenor (in powder form) was obtained from the University of Liege in Belgium and dandelion microspheres (ref TADL060206) were supplied by PARACHIMIC Company, Sfax, Tunisia.

2.3. Chemicals

All reagents used in the present study were of analytical grade. Lead (in the acetate form) was obtained from SD Fine Chemicals, Bhoisar, Mumbai, India. 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), L-Glutathione (reduced form), and all current chemicals were purchased from Sigma Chemical Co., (St. Louis, MO, USA).

2.4. Experimental procedure

2.4.1. Food preparation

Standard diet provided to the rats consisted of pellets containing a mixture of wheat, alfalfa, soybean, vitamins and minerals. Alternatively, a diet enriched with plants was prepared by mixing plant powder with food pellets in distiled water so as to obtain a homogenous paste. That mixture was cut into pellets and allowed to dry before starting the experiment. A preliminary study, using different plant doses in the diet (*i.e.* 0 to 15%, thus 0 to 150 g plant powder per kg of food pellets), did not reveal any toxic effects or oxidative stress in adult females treated with spirulina and dandelion at doses up to 2% and 5%, respectively. Higher doses resulted in the appearance of toxicity, diarrhoea and reduced growth, but were not lethal to rats.

2.4.2. Treatments

After one week acclimatisation in the laboratory conditions, adult females were placed with males on the proestrus night and the presence of spermatozoa in the vaginal smear was noted as day 0. Pregnant females (M, mothers) were individually housed in plastic cages in a temperature-controlled nursery (22–24 °C). Forty-eight pregnant rats were randomized into two sets of 24 rats. The first set consisted of control animals drinking distiled water. The second set was given water containing 6 g/L lead acetate, resulting in an average uptake of 5 mg lead/kg body weight/day (Ghorbel et al., 2001, 2002). Each group was then separated into three subgroups of eight animals. Rats belonging to C (control), S (spirulina) and D (dandelion) subgroups were given a normal diet, a diet enriched with spirulina (5%) or with dandelion (2%), respectively. Similarly, three subgroups treated with lead acetate were given either a normal diet (Pb), a diet enriched with spirulina (SPb) or a diet enriched with dandelion (DPb). All groups were treated from the 5th day of gestation to the 14th day of lactation, brain development being strongly sensitive to environmental pollutants during that period (Ronis et al., 1998; Bunn et al., 2001). At birth, pups from each mother were weighed and each litter was reduced to eight pups (4 males and 4 females) in order to maximise lactation performance (Fisheck and Rasmussen, 1987). During the lactating period, the dams' food and water intake was measured daily at the same time: the amount of ingested diet was calculated as the difference between the weight of feed placed one day (D_1) in the food bin and that remaining the day after (D_2). All the recorded data were then used to calculate the daily average feed intake over the whole experiment. Using that method, quantities of Pb, S and D ingested by each lactating dam were calculated from water and diet intake (data not shown).

2.5. Organ sampling

On day 14 after delivery, pups (control and treated rats) were anesthetized with chloral hydrate by intra-abdominal injection. The body weights of pups were recorded and blood samples were collected in heparin tubes by brachial artery. Plasma samples were drawn from blood after centrifugation at 2500 x *g* for 15 min. They were kept at -20 °C until analysis. The brain and cerebellum were drawn, cleaned and weighed. Some samples were homogenised (1:2, w/v) in 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl using an Ultra-Turrax device. Homogenates were centrifuged at 5000 x *g* for 25 min at 4 °C and aliquots of supernatant were kept at -30 °C until analyses. Other samples were immediately fixed into Bouin's solution (saturated picric acid added with 37–40% formaldehyde and glacial acetic acid, 75:25:5 v/v) for histological studies.

2.6. Evaluation of lead content

Mineralisation of blood and pellets was carried out at 200 °C in Kjeldahl tubes in the presence of a nitric acid/perchloric acid (2:1 v/v) mixture. Lead contents were then determined using a fast sequential atomic absorption spectrometer (220 FS-AA, Varian). Accordingly, no lead was detected in food pellets.

2.7. Determination of antioxidant enzymes activities and lipid peroxidation level

Levels of lipid peroxidation in brain and cerebellum were estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Yagi (1976).

Superoxide-dismutase (SOD) activity was determined in brain and cerebellum homogenate according to the method of Beyer and Fridovich (1987). Catalase (CAT) activity was measured using the method of Aebi (1984), and glutathione-peroxidase (GPX) activity was measured according to the method of Flohe and Gunzler (1984).

2.8. Protein quantification

Protein content was assayed as described by Lowry et al. (1951), using bovine serum albumin as standard.

2.9. Histological analyses

Small pieces of neonate rat brain and cerebellum were fixed in a Bouin's mixture, embedded in paraffin, and sectioned. The sections were stained with haematoxylin–eosin (Gabe, 1968) to examine tissue constitution. Then, lead deposits were evidenced by rhodizonate staining through characteristic dark brown colour (Tung and Temple, 1996).

2.10. Statistical analysis

The data were analysed using the statistical package program Stat Graphics plus 5.1 (stats graphics). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (FLSD) test as a post hoc test for comparison between groups. Differences were considered significant at different levels ($P < 0.05$, $P < 0.01$, $P < 0.001$).

3. Results

3.1. Effect of treatments on brain and cerebellum tissues

After staining with haematoxylin–eosin, 3 layers appeared in the cerebellum of newborn rats compared to mothers (Fig. 1A): an external granular layer (EGL), a molecular layer (ML) and internal granular layer (IGL). In cerebellum of rats from control mothers, the Purkinje cells were completely differentiated. Conversely, in rats from lead-intoxicated mothers, the cerebellum Purkinje cells had a round shape, were nucleated or even unnucleated, and lacked the axonal extensions which connect to the ML cells (Fig. 1B). Besides, ML cells appeared thinner than in controls. When mothers were intoxicated by lead and given a diet containing spirulina or dandelion, IGL structure, EGL thickness and ML development in neonate cerebellum (Fig. 1C and D) were close to those in control rats.

The rhodizonate staining revealed the presence of lead chelates in the cerebellum and brain of young rats born from mothers intoxicated with lead (Pb group) (Figs. 2B and 3B) while these chelates were absent in control rats (Figs. 2A and 3A). Addition