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The potential protective effect of green, black, red and white tea infusions against adverse effect of cadmium and lead during chronic exposure – A rat model study

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The protective effect of green (GT), black (BT), red (RT) and white (WT) tea infusions on the lungs, brains, hearts, livers and kidneys of adult Wistar rats exposed to Cd (7 mg/kg) and Pb (50 m/kg) was studied. The degree of reduction in the absorption of Cd and Pb in the organs compared to control group and the activity of SOD, CAT and GPx as well as GSH level was evaluated. It was determined that tea significant reduced the accumulation of Cd in the tissues. A significant reduction in the accumulation of Pb was recorded in the brain (WT), liver (GT, WT) and kidneys (BT, GT, RT, WT). A significant increase was observed in the activity of SOD, CAT and GPx in the organs of all rats from tea groups. It was found that the results obtained in rats receiving black, red and white tea were overall not worse than those recorded for rats receiving green tea. The obtained results suggest that drinking tea could be an effective method of reducing the adverse effect of environmental Cd and Pb pollution on the human body.

1. Introduction

The presence of toxic metals in food products has become a global problem. The most important source of toxic metals for man is food of plant origin, and in particular cereals (EFSA, 2010; Krajčovičová-Kudláčková et al., 2006), mostly with regard to the fact that they are the basis of nourishment throughout the world and are consumed most abundantly. Although, according to available literature, the content of cadmium (Cd) and lead (Pb) in food normally does not exceed standard levels (Sommar et al., 2013), due to the fact that these metals are capable of accumulating in tissues and have a long half-life (5–30 years for Cd and about 30 days for Pb in soft tissues and even up to 10 years for Pb in bones) (Castelli et al., 2005; Brito et al., 2005), their regular supply, even in small amounts, is hazardous. Exposure to Cd and Pb supplied with food is much more hazardous than with water (Winiarska-Mieczan, 2014).

Cd and Pb are quickly absorbed from the alimentary tract (Tong et al., 2000; Higazy et al., 2010). Glutathione GSH, as an important antioxidant synthesized in cells, is involved in detoxication of electrophilic xenobiotic through the formation of S-conjugates with toxic metabolites (Kerksick and Willoughby, 2005). Oxidative stress induced by Cd and Pb reduces the efficiency of the antioxidant defence system leading to damage in organs (Hamed et al., 2010). Antioxidant enzymes (e.g. superoxide dismutase SOD, catalase CAT, glutathione peroxidase GPx) decrease the toxicity of Cd and Pb (Tarasub et al., 2012). The use of exogenous antioxidants increases the total antioxidant capacity of the body and reduces the likelihood of inducing oxidative damage. From a practical nutritional point of view it is also important to examine food products containing significant amounts of antioxidant components in order to use them in a daily diet to prevent the hazardous effect of toxic metals on the human body. In available literature positive effects have been reported, among other things, for garlic (Padalko et al., 2012), honey (Abdel-Moneim and Ghafeer, 2007), rosemary (Abd El Kader et al., 2012), marjoram (Shati, 2011), kombucha tea (Ibrahim, 2013) and green tea (Hamed et al., 2010). Tea, as the most popular drink in the world apart from water, deserves particular attention (Hicks, 2009). According to some authors tea satisfies the conditions for classification as functional food (Wu and Wie, 2002). The most frequently drunk varieties are black and green tea (Hilal and Engelhardt, 2007).

Tea contains a number of polyphenols, such as, for example, tannic acid (Savolainen, 1992), catechins (Zaveri, 2006) and quercetin (Chen et al., 2009), substances with an antioxidant effect which are capable of chelating metals. Tannic acid administered

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orally to rats exposed to Cd and Pb protected their tissues (brains, hearts) against adverse effects of these metals, which was primarily manifested in increased SOD and CAT activity and reduced level of Cd and Pb in tissues (Winiarska-Mieczan et al., 2013; Winiarska-Mieczan, 2013). It was proved that catechins, including epigallocatechin-3-gallate (EGCG) (Khalaf et al., 2012; El-Shahat et al., 2009) and quercetin (Unsal et al., 2013) had a similar effect. Available literature comprises a large number of publications describing the protective effect of green tea extract in laboratory animals exposed to Cd or Pb (Hamed et al., 2010; Khalaf et al., 2012; El-Beltagy et al., 2015). However, no results are available concerning white tea being the richest source of antioxidants (Karori et al., 2007) and red tea which contains less of such compounds (Rusaczonek et al., 2010) but on the other hand has a documented anti-atherogenic effect (reducing the level of blood lipids) and prevents obesity (Hou et al., 2009). Also, no results are available concerning black tea – being the world's most popular and most abundantly drunk variety – despite it is a good source of antioxidants (Yashin et al., 2011).

Considering the worldwide popularity of tea and its beneficial properties, the present studies aimed to verify whether drinking tea regularly could prevent an adverse effect of toxic metals on the human organism during chronic exposure. Rats differ markedly from humans in many respects but rat models are most commonly used for studying human pathogenesis and diseases (Benito et al., 2011), toxic metals poisoning (Brzóska, 2012) as well as the efficacy of potential therapeutic interventions (Al-Rejaie et al., 2013). This study assumed that administering of black, green, red and white tea would decrease the accumulation of Cd and Pb and increase the activity of antioxidant enzymes (SOD, CAT, GPx) and GSH level in organs of rats.

2. Material and methods

2.1. Experimental animals

Adult male Wistar rats (12 weeks old, 322.7 ± 8.9 g) were purchased from the Centre of Experimental Medicine (Medical University of Białystok, Poland, No. 6/2003). Rats were housed one per polypropylene cages. The room was kept at 21 ± 3 °C, ensuring 55 ± 5% humidity and 12 h day–night cycle. This study was carried out in accordance with the statutory bioethical standards and was approved by the Ethics Committee at the University of Life Sciences in Lublin.

2.2. Treatments

The experiment involved 4 kinds of tea: green, black, red and white tea. To check whether adult rats would eagerly drink respective tea infusions an experiment was held in which rats were given the infusions (1 tea bag: 200 ml of distilled water at 90 °C for 5 min) as the only source of drink for 7 days (unpublished data). The experiment showed that rats were very reluctant to drink tea infusions (ca. 12 ml/24 h vs. ca. 25 ml/24 h in the group given drinking water). The consumption of feed did not exceed 10 g/day, and as a consequence about 15% reduction in body weight was recorded compared to the initial weight. The reason for low intake of infusions could be their excessively bitter taste not eagerly accepted by rats. Given such results the experiment was abandoned. Studies by certain authors showed that the bitter taste of beverages was due to tannic acid (Hagerman et al., 1998; Valentova et al., 2002), therefore an experiment was carried out to check at which concentrations water-based solutions of tannic acid would be most eagerly drunk by adult rats. The results of the experiment are presented elsewhere (Winiarska-Mieczan, 2013). The study demonstrated that rats drank 2% solutions of tannic acid most eagerly. In connection with these findings it was decided that experimental tea solutions will be diluted to contain tannic acid at 2%. The levels of Cd and Pb used in this study (7 mg of Cd and 50 mg of Pb) were based on the study of Brzóska et al. (2003, 2004), Martynowicz et al. (2004) and Kaczmarek-Wdowiak et al. (2004). Complex exposure to Cd and Pb was involved in the experiment since in the natural environment exposure to these factors is always parallel; whereas, the degree of absorption of these metals, and thus their toxicity is determined by the type of exposure (Winiarska-Mieczan, 2014). Since the beneficial effect of green tea on rats poisoned with Cd and Pb was demonstrated (Tarasub et al., 2012; Hilal and Engelhardt, 2007) in the experiment, in evaluating the effect of tea infusions on tissues rats receiving green tea were considered a reference group (positive control).

2.3. Experimental protocol

After 7 days of adaptation the rats were randomly assigned to 5 groups, each composed of 12 animals: DW – received distilled water; GT – received green tea infusion; BT – received black tea infusion; RT – received red tea infusion; WT – received white tea infusion. The rats were given *ad libitum* drinking fluids as a sole source of drink for 6 or 12 weeks and they were administered a standard diet mixed with 7 mg of Cd (as $CdCl_2$) and 50 mg of Pb (as $(CH_3COO)_2Pb$)/kg. The tea infusions were made by soaking tea bags purchased from a commercial source in distilled water at 90 °C for 5 min, in the proportion 1 tea bag: 200 ml of water. The tannic acid contents in 1000 ml of tea infusions were: 111 mg for green tea (China, Lipton), 94 mg for black tea (India, Lipton), 77 mg for red tea (China, Lipton), and 113 mg for white tea (China, Lipton). Total polyphenols content (expressed as tannic acid equivalent) in 1000 ml of tea infusions was: 2363 mg in green tea, 1220 mg in black tea, 996 mg in red tea, and 2668 mg in white tea. The infusions were divided into 30 ml samples which were frozen at −20 °C. The frozen samples were used every time to prepare solutions ensuring that the drinking solutions used throughout the term of the experiment had identical tannic acid concentration. The control of feed consumption was recorded weekly. Feed was replaced every week, and fluids every two days (tea solutions were prepared freshly). 24 h after the last dose of food and fluids, both after 6 and 12 weeks of the experiment, 6 rats from each group were subjected to light CO_2 anaesthesia and killed by their spinal cord being broken. The lungs, brains, hearts, livers and kidneys were excised immediately as a whole and washed with ice-cold physiological saline (0.9% NaCl). Next, they were packed separately in plastic bags and kept frozen at −20 °C until Cd and Pb levels were measured.

2.4. Chemical analysis

After dry mineralisation (muffle furnace, 450 °C, 12 h, oxidant – H_2O_2) of the samples the ash was dissolved in 10 ml of 1 M nitric acid (HNO_3). A Varian Spectr AA 880 atomic absorption spectrometer equipped with graphite furnace and transverse Zeeman background correction was used for measurements of Cd ($\lambda = 228.8$ nm, lamp current 4 mA, spectral band pass 0.5 nm, LOD 0.001 mg/kg, LOQ 0.004 mg/kg, recovery 96%) and Pb (217.0 nm, lamp current 10 mA, spectral band pass 1 nm, LOD – 0.011 mg/kg, LOQ – 0.03 mg/kg, recovery 95%). Argon was used as the pure gas. All samples were analyzed in duplicate, and the deviation of measurement was below 5%. The quality assurance procedure involved blank samples (1 M HNO_3) and CRM-185R certified reference material (bovine liver). The method was described previously (Winiarska-Mieczan et al., 2013). The supernatant for