Experimental and Toxicologic Pathology 67 (2015) 331–339

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Hematological and serum biochemical parameters of blood in adolescent rats and histomorphological changes in the jejunal epithelium and liver after chronic exposure to cadmium and lead in the case of supplementation with green tea vs black, red or white tea

Ewa Tomaszewska[a,*], Anna Winiarska-Mieczan[b], Piotr Dobrowolski[c]

[a] Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, 20-950 Lublin, Poland
[b] Department of Bromatology and Food Physiology, University of Life Sciences in Lublin, 20-950, Poland
[c] Department of Comparative Anatomy and Anthropology, Maria Curie-Skłodowska University, 20-033 Lublin, Poland

ARTICLE INFO

Article history:
Received 29 November 2014
Accepted 26 February 2015

Keywords:
Cadmium
Lead
Tea
Rat
Jejunum
Liver
Histomorphometry
Blood parameters

ABSTRACT

Rats were used to check whether regular consumption of black, red, or white tea would have a protective effect similar to the action of green tea on the intestine and liver in the case of exposure to Cd and Pb within the limits of human environmental exposure to these elements. Rats at the age of 6 weeks were divided into the control and four groups supplemented with green (GT), black (BT), red (RT), or white (WT) tea extracts. Their diet (except the control) was mixed with 7 mg Cd/kg and 50 mg Pb/kg. The experiment lasted 12 weeks. The effects of administration of tea in Cd- and Pb-poisoned rats on plasma biochemical parameters and the jejunal epithelium and liver were determined. The highest body mass was found in the GT group. The highest hemoglobin and Fe concentrations were in the control and GT groups. The highest activity of AST was in groups poisoned with Cd and Pb independently on supplementation. The highest ALT activity was in BT and RT groups with lower content of polifenoles. Pb and Cd disturbed the liver leading to necrosis and fatty degenerative changes, and a loss of normal architecture of the hepatocytes. Rats from the GT group had the highest cell proliferation rate in intestinal glands and the largest absorptive surface. Black, red, and white tea exerted a varied impact on the histological structure and innervation of the small intestine wall as well as on the absorptive function of small intestine mucosa in rats poisoned with Pb and Cd than green tea. On the other hand, taking into account the number of apoptotic cells, the effect of the teas was the same. Moreover, it is clear that long term exposure to Cd and Pb contamination causes toxic effect in the liver.

1. Introduction

Tea is the second most commonly consumed beverage in the world, after water (Wu and Wei, 2002). Apart from Sri Lanka and Kenya, China and India are the leading consumers and at the same time the world's largest exporters of tea (Hicks, 2009). The most frequently drunk varieties are black and green tea (Hilal and Engelhardt, 2007). The popularity of tea is determined by its healthy properties connected with the presence of antioxidants, mainly polyphenols,[1] thanks to which tea can be classified as functional food (Wu and Wei, 2002). Polyphenols account for 25–35% of the dry matter of tea leaves (Chaturvedula and Prakash, 2011). Drinking tea prevents, among other conditions, obesity, diabetes, tumors, cardiovascular diseases, and damage to organs due to oxidative stress (Chung et al., 1998; Yang and Landau, 2000; Toschi et al., 2000; Priyadarshi et al., 2003; Wu et al., 2003; Davies et al., 2003; Arteel et al., 2005; van Dieren et al., 2009; Abolfathi et al., 2012; Lu et al., 2012; Fuhrman et al., 2013; Chen and Zhang, 2013; Mähler et al., 2013). Infusions and extracts of black, green and white tea were found to have strong antibacterial properties (Almajano et al., 2008; Flayyih et al., 2013; Radji et al., 2013).

It was found that tea infusions enhanced detoxification processes in rats by binding toxic metals, inhibiting absorption, and intensifying excretion thereof from the body (Kim and Rhee, 1994; Khalaf et al., 2012). This is particularly important when the contamination of the human environment, including food, with metals is a global issue (EFSA, 2012a, 2012b). Most scientists have

* Corresponding author. Tel.: +48 81 4456973; fax: +48 81 4456963.
E-mail address: ewaRST@interia.pl (E. Tomaszewska).

[1] The tea is the most popular and universal beverage in the world. This phenomenon is determined by its properties linked with polyphenols. So that tea can be included into functional food.

http://dx.doi.org/10.1016/j.etp.2015.02.005
0940-2993/

studied the properties of green tea, which is particularly rich in polyphenols having a very strong chelating effect and effectively reducing the toxicity of metals to organisms by increasing the antioxidant potential in tissues (Khalaf et al., 2012).

The increase of the antioxidant potential prevents the occurrence of oxidative stress in cells, which is the cause of their failure. Particularly vulnerable are liver cells (Akintunde and Oboh, 2012). Moreover, organic compounds of Pb are metabolized in the liver to strongly neurotoxic compounds such as triethyl lead and thimethyl lead (Stewart et al., 2002). There is no data about histopathological changes in liver or intestine in animals exposed to Cd or Pb. One study on fishes shows, that heavy metals can either increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on type and concentration, fish species, length of exposure and other factors (Das and Gupta, 2013).

Cadmium (Cd) and lead (Pb) are toxic metals commonly occurring in the human environment. The environmental human exposure to Cd is approx. 5 mg/kg of body weight/week and to Pb – ca. 35 mg/kg of body weight/week (Kaczmarek-Wdowiak et al., 2004). In 2012, EFSA changed the tolerable intake levels for Cd and Pb, established the TWI (Tolerable Weekly Intake) for adults and children at 2.5 μg Cd/kg of body weight/week, and determined two values of the BMDL (Benchmark Dose Lower Confidence Limit): $BMDL_{10}$ (neurotoxic effect) – 0.63 μg Pb/kg of body weight/day for adults and $BMDL_{10}$ – 0.5 μg/kg of body weight/day for children (EFSA, 2012a, 2012b). Humans get to toxic metals through the respiratory system, gastrointestinal tract, and small amounts through the skin (Godt et al., 2006). The degree of the absorption of the toxic metals from the gastrointestinal tract depends on the chemical form and is relatively low. However, when commercially available foods is derived from the whole word, also areas with a higher concentration of these metals in the environment (air, soil, water), food becomes a very serious source of these metals for humans. The most important source of Cd and Pb in the human diet are plant products, particularly cereals and their products, because they are basic food products, consumed in the largest quantities (EFSA, 2012a, 2012b).

Rats are not perfect model animals for man but they are most frequently used for testing of the effects of agents that are toxic or potentially hazardous to humans. This also refers to the toxicity of metals and a possible preventive and therapeutic effect (Walton, 2007; Brzóska, 2012; Brzóska et al., 2012; Al-Rejaie et al., 2013).

The rat model was used to check whether regular consumption of black, red or white tea would have similar protective effect to the action of green tea on the intestine and liver in the case of exposure to Cd and Pb within the limits of human environmental exposure to these elements.

The aim of this study was to examine whether other types of teas will also be effective: white – because it has more antioxidants than green (Hilal and Engelhardt, 2007), black – because the most popular in Poland and in the world, red – also have a lot of antioxidants (Almajano et al., 2008; Hicks, 2009; Chaturvedula and Prakash, 2011).

2. Materials and methods

2.1. Ethics statement

The experimental procedures used throughout this study were approved by the Local Ethics Committee on Animal Experimentation of University of Life Sciences of Lublin, Poland. All experiments complied with the Guiding Principles for Research Involving Animals. All efforts were made to minimize the number of animals used as well as their suffering.

Table 1
Measurement parameters for the determination of Pb and Cd.

	Pb	Cd
Wave length [nm]	217.0	228.8
Lamp current [mA]	10	4
Spectral band pass [nm]	1	0.5
LOD [mg/kg]	0.011	0.001
LOQ [mg/kg]	0.03	0.004
Pure gas	Argon	Argon
Background correction	Zeeman	Zeeman
Mean recovery rate [%]	95	96
The deviation of duplicate measurement [%]	5.2	5.0

2.2. Animals and basal diet

The experiment lasted 12 weeks. Forty-eight adolescent male Wistar rats at the age of 6 weeks, weighing 210.6 ± 12.1 g were used. All the animals were weighed and observed in individual polypropylene cages (the dimensions of 380 mm × 200 mm × 590 mm) and acclimated in the laboratory for 7 days. The rats were kept in a room at a temperature of 21 ± 3 °C, humidity of 55 ± 5%, and a day cycle 12 h:12 h was maintained. The rats had free access to water and fed ad libitum with common laboratory animal feed mixed with 7 mg Cd/kg and 50 mg Pb/kg. The rats were weighted every 7 days. The weekly intake of feed provided the basis for the calculation of the average weekly supply of Cd and Pb (Table 1). Fresh feed was provided every 7 days. The feed was prepared in the laboratory by adding water-based solutions of Cd (as $CdCl_2$) and Pb (as $(CH_3COO)_2Pb$) to ground standard feed which was later carefully mixed and granulated by mechanical methods. The level of metals supplied in the feed (7 mg for Cd/kg and 50 mg for Pb/kg) was calculated to ensure that the daily supply of Cd and Pb did not exceed the environmental exposure of humans.

It is known that green tea as well as its active ingredients has very good protective influence on organisms exposed to Cd and Pb. Moreover, adverse effects of Cd and Pb on the living organism and its various organs are known as well. For these reasons, the number of experimental animals has been limited to four groups to minimize the number of animals used in the study.

Animals were randomly divided into the control group as a references group (without Cd, Pb and teas; n = 12) and four groups subjected to the supplementation of different kind of tea extract – green tea (GT; n = 12), black tea (BT; n = 12), red tea (Pu-erh; RT; n = 12), and white tea (WT; n = 12).

On the basis of the available literature, it was found that the preventive effect of green tea (served in various concentrations and for different lengths) on laboratory animals exposed to toxic metals has been proven (Hamden et al., 2008). Accordingly, in the present experiment, it was assumed that the group of rats treated with green tea can be considered as a positive control in order to check whether other types of tea also will act protectively, because information on this subject is not available (white and red teas) or very poor (black tea).

Fresh drinking solutions were provided every two days. After 12 weeks the rats were starved for 24 h and weighted. Afterwards, rats were euthanized by CO_2 inhalation. Immediately after euthanasia, blood was sampled directly from the rats' heart into 6 ml Vacutest vacuum tubes with heparin-Li as a coagulant. The liver also was isolated and weighted. Relative liver mass (%) was calculated as a ratio of liver weight and body weight.

2.3. Preparation of tea solutions

Teas used in the experiment were purchased from a commercial source. The brew of black tea (India, Lipton), green tea