Experimental and Molecular Pathology 103 (2017) 26–32

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Investigation of the effects of *Lycium barbarum* polysaccharides against cadmium induced damage in testis

Maria Vittoria Varoni, Sergio Domenico Gadau[1], Valeria Pasciu[1], Elena Baralla*, Elisa Serra, Domenico Palomba, Maria Piera Demontis

Department of Veterinary Medicine, University of Sassari, Sassari, Italy

ARTICLE INFO

Keywords:
Cadmium
Testis
Lycium barbarum polysaccharide
Oxidative stress
3-nitro-L-tyrosine

ABSTRACT

This study describes the effects of *Lycium barbarum* polysaccharides (LBP) on testicular damage induced by cadmium (Cd). Adult male rats were i.p. injected with $CdCl_2$ (4 mg/Kg, once) with or without LBP pretreatment (300 mg/Kg orally, once a day, for 30 days). Testis weight, morphological/histological structure and oxidative stress parameters were evaluated. Several adverse effects were observed after $CdCl_2$ injection, with a significant decrease in body/testis weight ratio ($P < 0.05$), gross morphological changes with hyperemia of the parenchyma, increased volume and alteration in the structure of the seminiferous tubules. Furthermore, Cd intoxication caused a significant decrease of glutathione (GSH) and Trolox equivalent antioxidant capacity (TEAC) in testis ($P < 0.05$) together with a significant increase ($P < 0.01$) of 3-nitro-L-tyrosine (3NT) while malondialdehyde (MDA) did not change. LBP pretreatment caused slight signs of improvement in the morphology of the seminiferous tubules. Our results confirm that Cd induces testicular damage and suggest the oxidative stress involvement. LBP could ameliorate Cd testicular damage but further investigations are needed.

1. Introduction

Cadmium (Cd) is an heavy metal widely distributed in the environment and released primarily from electroplating, battery manufacturing, plasting and fertilizers (Stohs and Bagchi, 1995). Apart from the inhalation of Cd particles or fumes from occupational exposure (McKenna et al., 1997), the main exposition to Cd occurs through contaminated food, drinking water and tobacco smoking (Goering et al., 1994; Waalkes, 2003). Cd has a long half-life (20–30 years) and can accumulate in many organs such as liver, kidney and testis causing many morphological and pathological changes (Thompson and Bannigan, 2008; Obianime and Roberts, 2009; Lakshmi et al., 2012; Sarkar et al., 2013). Testes are particularly sensitive to Cd and environmental exposure has been associated with male infertility (Xu et al., 2003) and reduced sperm quality (Pant et al., 2003) in humans. Testicular damage, after Cd administration, has been observed in different animal models at different stages of growth and maturity. The associated testicular lesions, in experimental animals, include severe hemorrhage, edema, necrosis that result in the total disruption of the seminiferous tubules (Shiraishi and Waalkes, 1996). It is well known that the main mechanism of Cd toxicity could be mediated by oxidative-stress, a condition where there is an imbalance between generation and elimination of reactive oxygen species and reactive nitrogen species (ROS/RNS) such as nitric oxide, peroxynitrite, and 3-nitro-L-tyrosine (3NT). The enhanced production of oxygen free radicals may contribute to increase lipid peroxidation and reduce antioxidant defense system (Ognjanovic et al., 2010; Santos et al., 2004; Wang et al., 2015). The production of reactive oxygen species (ROS) could lead to testicular damage through oxidation of the cell membranes in testes, which are susceptible to peroxidation injury (Lee et al., 2009). To protect the integrity of cells from oxidative processes caused by free radicals, animals have enzymatic and non-enzymatic defense mechanisms that include superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Different antioxidants were effective in reducing Cd-induced testes damage. Recently many natural antioxidants have been used in the treatment of various diseases. *Lycium barbarum* berries (also commercially named goji berry) have been used in China and other Asian countries as medicinal herb for > 2000 years. They have long been used to promote fertility and as potent anti-aging and antioxidant agent (Cheng et al., 2015). *Lycium barbarum* polysaccharides – (LBP), proteoglycans contained in the fruit, have been extensively studied and they seem to be the most responsible for the pharmacological activity of *Lycium barbarum.*

LBP exhibit a wide array of therapeutic effects on aging, cancer,

* Corresponding author.
E-mail address: ebaralla@uniss.it (E. Baralla).
[1] Sergio Domenico Gadau and Valeria Pasciu contributed equally to this work.

http://dx.doi.org/10.1016/j.yexmp.2017.06.003
Received 19 September 2016; Received in revised form 15 May 2017; Accepted 16 June 2017
Available online 20 June 2017

atherosclerosis, diabetes, hepatitis and male infertility in different animal models (Cheng et al., 2015). Recently, many authors have focused on LBP protective effects on reproductive system in animals against testicular damage induced by heat exposure, local irradiation and chemical substances. This protective effect is, at least in part, attributed to antioxidation, promotion of cell proliferation, and anti-apoptosis (Luo et al., 2006; Luo et al., 2014; Xin et al., 2012; Zhang et al., 2013). At the moment, to our knowledge, the effect of LBP on Cd induced testicular damage is not yet studied. Therefore, we believe that it is interesting to investigate if LBP may have protective effects against Cd-induced negative changes on histological structure of testes and oxidative stress in male rats. Thus, histopathological changes and oxidant and antioxidant status of testicular tissue were evaluated by measuring malondialdehyde (MDA), 3-nitro-L-tyrosine (3NT), SOD, GSH and Trolox equivalent antioxidant capacity (TEAC) in plasma and testes.

2. Material and methods

2.1. Drugs and chemicals

Lycium barbarum fruits powder containing 40% of polysaccharides (LBP) was kindly provided by Specchiasol (Verona, Italy). The powder was dissolved in tap water and LBP (300 mg/Kg) administered once a day by gavage. Cadmium chloride ($CdCl_2$) was purchased from Sigma-Aldrich (Milan, Italy) and dissolved in saline before administration (4 mg/Kg). All other chemicals and reagents used were of the highest commercial quality and obtained from Sigma-Aldrich (Milan, Italy). Dose, route and duration of administration of LBP and Cd were chosen following data yet reported in literature (Cheng and Kong, 2011; Iscan et al., 2002).

2.2. Animals and experimental groups

Male Wistar rats, weighing 250 ± 30 g, were obtained from Envigo (Udine, Italy). Animals were kept at standard housing conditions (temperature 22–24 °C, humidity (60 ± 5%), 12 h light/dark cycle) and supplied with standard laboratory chow (Mucedola, Milan, Italy) and drinking water ad libitum. The experimental protocol was approved by the Italian Minister of Health and all animal experiments were performed according to the Directive 2010/63/EU.

After an adaptive period of one week, animals were randomly divided into four experimental groups (5 rats/group).

The first group (control, CTRL group) received tap water for thirty days by gastric gavage and then a single i.p. injection of normal saline (vehicle of cadmium chloride) on the 30th day of the experiment.

The second group (Cd group) was pretreated with tap water for thirty days by gastric gavage and then i.p. injected once with cadmium chloride (4 mg/Kg) on the 30th day of the experiment.

The third group (LBP group) received LBP (300 mg/Kg) dissolved in tap water for thirty days by gastric gavage and then a single i.p. injection of normal saline on the 30th day of the experiment.

Finally the fourth group of animals (LBP + Cd group) was pretreated with LBP (300 mg/Kg) dissolved in tap water for thirty days by gastric gavage and then i.p. injected once with cadmium chloride (4 mg/Kg) on the 30th day of the experiment.

2.3. Collection of samples

Twenty-four hours after cadmium/saline administration, rats were weighed and deeply anesthetized by i.m. injection of zoletil (25 mg/Kg) plus xilazine (1 mg/Kg). After sacrifice testes were removed quickly from animals, washed on ice-cold normal saline, dried with filter paper and weighed. One testis was used to determine the oxidative stress parameters and the other for histology and immunofluorescence staining.

Table 1
Body/testis weight in CTRL, Cd, LBP and LBP + Cd groups. All data are expressed as means ± SD; $n = 5$ rats.

	CTRL group	Cd group	LBP group	LBP + Cd group
Body weight (g)	338 ± 15	340 ± 13	342 ± 28	345 ± 17
Testis weight (g)	1.62 ± 0.3	2.3 ± 0.7	1.84 ± 0.1	2.32 ± 0.2*
Body/testis weight	215 ± 47	155 ± 30*	187 ± 19	149 ± 13*

* $P < 0.05$ when compared to the CTRL group.

2.4. Histology

Left testis belonging to each experimental group was collected and divided into two halves. One has been fixed in 4% buffered neutral formalin. After fixation, tissues were processed using graded ethanol series and resized into thinner pieces. These samples were then embedded in paraffin blocks. Sections of about 10 mm thickness were cut, stained with hematoxylin and eosin (H & E) by the standard method and examined under light microscope. The other half was processed for immunofluorescence staining.

2.5. Sample preparation

A precipitating solution containing *N*-ethylmaleimide (NEM), a derivatizing agent to prevent the excessive oxidation of GSH, was prepared to a final concentration of 20 mM NEM, 2% sulfosalicylic acid and 2 mM EDTA in 15% methanol as described by Moore et al. (2013).

Testes were collected and, after being weighed, were transferred into tubes containing polyphosphate buffer (PBS) to wash them from the excess of blood. Clean testes were treated with a solution containing PBS-Triton × 100 and then homogenized as described by Pasciu et al. (2015). Obtained samples were collected and stored at − 80 °C until use for the MDA, TEAC and SOD assays. To assess GSH in testes, 100 µl of supernatant was treated without being frozen and added to 400 µl of the precipitating solution (above described), vortexed and allowed to incubate at room temperature for 45 min in order to obtain the complex GSH-NEM. After a centrifugation step (14,000*g*, 5 min, + 4 °C) the obtained supernatant was transferred into polypropylene tubes and stored at − 80 °C until analysis.

2.6. Oxidative stress markers

2.6.1. Malondialdehyde assay

MDA, one of the several low-molecular-weight end-products of lipid peroxidation, was evaluated by spectrophotometric detection according to the thiobarbituric acid-reactive substances (TBARS) assay described by Spanier and Traylor (1991) with some modifications. In detail 100 µl of sample were thawed and added to 100 µl of 33% glacial acetic acid, 75 µl of 10% sodium dodecyl sulphate (SDS), 100 µl of 50 mM Tris-HCl pH 7.4 and 250 µl of 0.75% thiobarbituric acid (TBA). The mixture was then incubated for 1 h at 100 °C and immediately cooled on ice. After 10 min, 200 µl of 33% acetic acid were added and samples were centrifuged for 20 min at 7000*g*. The supernatant absorbance was then read with a Thermo Electron Corporation Genesys 10UV spectrophotometer at 535 nm. MDA values were calculated using a standard curve and expressed as nmol of MDA formed per gram wet weight of tissue.

2.6.2. Reduced glutathione

A liquid chromatographic system with triple quadrupole mass spectrometry detection (LC-MS/MS) was used to measure GSH in whole blood and testes using thiosalicylic acid (TSA) as internal standard (IS) as described by Varoni et al., 2016.

GSH values in samples were calculated using a standard curve built in 15% methanol (range from 10 to 10,000 ng/ml) and expressed as