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Nordihydroguaiaretic acid attenuates potassium dichromate-induced oxidative stress and nephrotoxicity

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Abstract

Larrea tridentata also known as Creosote bush, Larrea, chaparral, greasewood or gobernadora has been used in the folk medicine for the treatment of several illnesses. The primary product that is present at high concentrations in the leaves from this plant is nordihydroguaiaretic acid (NDGA) which is a powerful antioxidant. On the other hand, potassium dichromate ($K_2Cr_2O_7$)-induced nephrotoxicity is associated with oxidative stress. The aim of this work was to study the effect of NDGA on $K_2Cr_2O_7$-induced nephrotoxicity and oxidative stress. Nephrotoxicity was induced by a single injection of $K_2Cr_2O_7$ (15 mg/Kg). A group of $K_2Cr_2O_7$-treated rats was administered NDGA by mini osmotic pumps (17 mg/Kg/day). The results show that NDGA was able to ameliorate the structural and functional renal damage evaluated by histopathological analysis and by measuring proteinuria, urinary excretion of *N*-acetyl-β-D-glucosaminidase, serum creatinine, and serum glutathione peroxidase activity. In addition, immunostaining of 4-hydroxy-2-nonenal and 3-nitrotyrosine, markers of oxidative and nitrosative stress, respectively, was ameliorated by the NDGA treatment. These data strongly suggest that the antioxidant properties of NDGA are involved in its renoprotective effect in $K_2Cr_2O_7$-treated rats.

Keywords: *Larrea tridentata*; Antioxidant; Nephrotoxicity; Oxidative stress; Potassium dichromate

1. Introduction

Larrea tridentata belongs to the family Zygophyllaceae and also is known as Creosote bush, Larrea, chaparral, greasewood or gobernadora, which dominates some areas of the desert southwest in the United States and Northern Mexico, as well as some areas of Argentina (Arteaga et al., 2005). Chaparral tea has been used in the folk medicine for the treatment of more than 50 ailments including rheumatism, arthritis, diabetes, gallbladder and kidney stones, and inflammation (Arteaga et al., 2005). This tea has antioxidant properties (Zang et al., 1999). In addition, *L. tridentata* is a notable source of natural products with approximately 50% of the leaves dry weight as extractable matter. The resin that covers the leaves yields 19 flavonoid aglycones, as well as several lignans, notably including nordihydroguaiaretic acid (4-[4-(3,4-dihydroxyphenyl)-2,3-dimethylbutyl]benzene-1,2-diol, NDGA) (Arteaga et al., 2005). NDGA is a recognized antioxidant and more

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recently, it has been demonstrated that NDGA is a potent *in vitro* scavenger of peroxynitrite ($ONOO^-$), singlet oxygen, hydroxyl radical ($OH^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), and hypochlorous acid (Floriano-Sanchez et al., 2006). The antioxidant effect of NDGA has been observed in renal and hepatic toxicity induced by ferric-nitrilotriacetate (Ansar et al., 1999), ozone induced tyrosine nitration in lungs (Floriano-Sanchez et al., 2006), and streptozotocin-induced diabetic nephropathy (Anjaneyulu and Chopra, 2004). Ansar et al. (1999) found that NDGA was able to prevent the increase in renal and hepatic lipid peroxidation and H_2O_2 generation and the decrease in renal and hepatic glutathione (GSH) content and GSH-S-transferase, GSH reductase, glucose-6-phosphate dehydrogenase and catalase activities induced by ferric-nitrilotriacetate. Furthermore, NDGA treatment was able to prevent ozone-induced tyrosine nitration in lungs (Floriano-Sanchez et al., 2006). In addition, Anjaneyulu and Chopra (2004) found that NDGA prevented the increase in renal malondialdehyde levels and the decrease in renal GSH content and in superoxide dismutase and catalase activities induced by streptozotocin in rats. Furthermore, it has been found that NDGA has several health beneficial properties including: (a) inhibition of the growth of several human cancer types both *in vitro* and *in vivo* (Huang et al., 2004; Hofmanova et al., 2002), (b) chemopreventive ability in models of carcinogenesis (Moody et al., 1998; Ansar et al., 1999), (c) degradation of preformed Alzheimer's β-amyloid fibrils *in vitro* (Ono et al., 2002), and (d) protection of cultured rat hippocampal neurons against the toxicity of amyloid β-peptide (Goodman et al., 1994), interrupting a neurodegenerative pathway relevant to the pathophysiology of Alzheimer's disease. On the other hand, potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems (Barceloux, 1999). The oxidation state and solubility of chromium compounds determine their toxicity. In contrast to Cr^{3+}, which is a naturally occurring form and an essential trace element for humans and other mammals, Cr^{6+} compounds are highly toxic (Wang et al., 2006). $K_2Cr_2O_7$ is a hexavalent form of Cr and has been demonstrated to induce oxidative stress and carcinogenic in nature (Stohs and Bagchi, 1995; Norseth, 1981; Von Burg and Liu, 1993; Bagchi et al., 2002). The kidney is the principal route of chromium excretion and it has been reported that acute exposure induces an increase in chromium kidney content on $K_2Cr_2O_7$-treated rats (Pedraza-Chaverri et al., 2005). Exposition to Cr^{6+} produced anatomical lesions at the level of the proximal tubular cells (Franchini et al., 1978) and lipid peroxidation in human kidney (Huang et al., 1999). Interestingly, evidences suggest that reactive oxygen species (ROS) are involved in Cr^{6+}-induced cell injury (Liu and Shi, 2001; Bagchi et al., 2002; Travacio et al., 2001). Chromium reduction intermediates (Cr^{5+} and Cr^{4+}) may be toxic as they involve ROS production (Stohs et al., 2000; Shi and Dalal, 1990, 1994), which may be generated during physiological conditions. *In vitro*, hydrogen peroxide (H_2O_2)-induced Cr^{6+} reduction has been shown to produce $OH^{\cdot}$ via a Fenton-like reaction (Aiyar et al., 1991; Shi and Dalal, 1990; Tsou et al., 1996). *In vivo* experiments have shown that $K_2Cr_2O_7$ exposition induces renal oxidative and nitrosative stress measured as protein carbonyl content and 3-nitrotyrosine (3-NT) immunostaining, respectively (Barrera et al., 2003; Pedraza-Chaverri et al., 2005). The role of oxidative stress in the renal damage induced by $K_2Cr_2O_7$ has been supported additionally by the fact that some antioxidants such as α-tocopherol, ascorbic acid, and GSH (Appenroth and Winnefeld, 1998; Arreola-Mendoza et al., 2006; Na et al., 1992; Sugiyama, 1992; Hojo and Satomi, 1991) and the previous induction of heme oxygenase-1 (HO-1) (Barrera et al., 2003) are able to ameliorate $K_2Cr_2O_7$-induced nephrotoxicity and oxidative damage. To our knowledge, the potential protective effect of NDGA on $K_2Cr_2O_7$-induced nephrotoxicity has not been explored. Based on the above information, the hypothesis was made that NDGA may reduce $K_2Cr_2O_7$-induced renal injury. The aim of this study was to examine the effect of NDGA on $K_2Cr_2O_7$-induced nephrotoxicity and oxidative and nitrosative stress.

2. Materials and methods

2.1. Reagents and materials

Dimethyl sulfoxide (DMSO), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, NDGA, NADPH, GSH, and GSH reductase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Commercial kits for the measurement of creatinine concentration (Sera-pak plus creatinine) were from Bayer (Tarrytown, NY, USA). Mouse monoclonal anti-4-hydroxy-2-nonenal (4-HNE) antibodies (Cat. # 24325) were purchased from Oxis International, Inc. (Portland, OR, USA). Mouse monoclonal antibodies anti-3-NT (Cat. # 189542) were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). The secondary antibodies biotin SP-conjugated AffiniPure donkey anti-mouse IgG (Cat. # 715-065-151) were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). H_2O_2 was purchased from Mallinckrodt Baker (Xalostoc, Mexico). Declere was from Cell Marque (Hot Springs, AR, USA). ABC-kit Vectastain was from Vector Laboratories (Orton Southgate, Peterborough, UK). Diaminobenzidine substrate (Cat. # K3466) and Mayer's Hematoxylin (Lillie's Modification) (Cat. # S3309) were from DAKO Corporation (Carpinteria, CA, USA). Sodium pentobarbital was from Pzifer (Mexico). All other chemicals were reagent grade and commercially available. Alzet miniosmotic pumps Model 2004 (mean fill volume of 200 μL and mean pumping rate of 0.25 μL/h) were from Durect Corporation (Cupertino, CA, USA). Stainless steel metabolic cages were from Allentown Caging Equipment Co. (Allentown, NJ, USA).

2.2. Experimental design

Experimental work followed the guidelines of Norma Official Mexicana Guide for the use and care of laboratory animals (NOM-062-ZOO-1999) and for the disposal of biological residues (NOM-087-ECOL-1995). Fifty two female Wistar rats bred-in house (200–230 g) were used. Animals were provided with a standard commercial rat chow diet (Harlan Teklad Global diet 2018S sterilized, Harland Teklad, Madison, WI, USA) and water *ad libitum*. Housing room was maintained under constant condi-