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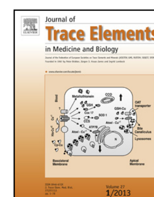

Nutrition

# Purple carrot extract protects against cadmium intoxication in multiple organs of rats: Genotoxicity, oxidative stress and tissue morphology analyses

Samuel Rangel Claudio[a], Andrea Pittelli Boiago Gollucke[a], Hirochi Yamamura[a], Damila Rodrigues Morais[b], Giovana Anceski Bataglion[b], Marcos Nogueira Eberlin[b], Rogerio Correa Peres[a], Celina Tizuko Fujiyama Oshima[c], Daniel Araki Ribeiro[a,*]

[a] Department of Biosciences, Federal University of Sao Paulo, UNIFESP, SP, Brazil
[b] ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas (UNICAMP), Campinas, SP, Brazil
[c] Department of Pathology, Paulista Medical School, Federal University of Sao Paulo, UNIFESP, SP, Brazil

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ABSTRACT

The aim of this study was to investigate if purple carrot extract is able to protect against the noxious activities induced by cadmium exposure in multiple organs of rats. For this purpose, histopathological analysis, genotoxicity and oxidative status were investigated in this setting. A total of twenty Wistar rats weighing 250 g on the average, and 8 weeks age were distributed into four groups ($n = 5$), as follows: Control group (non-treated group, CTRL); Cadmium group (Cd) and Purple carrot extract groups at 400 mg/L or 800 mg/L. Histopathological analysis revealed that liver from animals treated with purple carrot extract improved tissue degeneration induced by cadmium intoxication. Genetic damage was reduced in blood and hepatocytes as depicted by comet and micronucleus assays in animals treated with purple carrot extract. SOD-CuZn and cytocrome C gene expression increased in groups treated with purple carrot extract. Purple carrot extract also reduced the 8OHdG levels in liver cells when compared to cadmium group. Taken together, our results demonstrate that purple carrot extract is able to protect against cadmium intoxication by means of reducing tissue regeneration, genotoxicity and oxidative stress in multiple organs of Wistar rats.

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## 1. Introduction

Nowadays, carrots (*Daucus carota* L.) are considered the 10 most economically important vegetable crops grown worldwide [1]. Carrot cultivars appear in five taproot color types: purple, orange, yellow, red, and white. In general, orange carrots contain high amounts of α-and β-carotene; yellow carrots contain lutein, the red color of carrots is due to lycopene, while polyphenol substances, mostly anthocyanins are typical for purple roots [2]. In the last years, purple carrots (*D. carotas* sp. sativus var. atrorubens Alef.) are increasing popularity, because they contain high amounts of anthocyanin in their flesh taproots [3]. Anthocyanins from purple carrots are commonly used as natural food colorants in candies, ice cream, and beverages. This is because they remain stable when exposed to heat and light, and have increased pH values [4]. Purple carrots also contain some trace amounts of peonidin- or pelargonidin-based anthocyanins in their taproots [5].

Cadmium is a toxic heavy metal extremely harmful to humans and other mammalian species. It is present in air, soil, sediments, water and smoking. After intake, cadmium accumulates in multiple organs and tissues, particularly in liver and kidneys [6]. Long-term or even short term exposure leads to a wide range of noxious health effects, including renal dysfunction, cardiovascular disease, hypertension, osteoporosis, hepatotoxicity, pancreatic activity changes and cancers in many organs [7,8]. For this reason, International Agency for Research on Cancer has categorized cadmium as carcinogenic to humans and animals [9]. Unfortunately, human exposure to cadmium is increasing in the next decades, mainly in developing countries due to rapidly growing industries with increasing consumption and subsequent release into the environment.

* Corresponding author at: Departamento de Biociências Universidade Federal de São Paulo—UNIFESP, Av. Ana Costa 95, Zip code: 11060-001, Santos, SP, Brazil. Fax: +55 13 38783823.
*E-mail address:* daribeiro@unifesp.br (D.A. Ribeiro).

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Several underlying mechanisms at cellular or even molecular levels have been purposed for cadmium toxicity. Cadmium is involved in the disruption of many genomic processes, the mechanisms of which are being gradually understood so far. It has been established that oxidative stress plays a crucial role for cadmium toxicity. Following the intracellular contact of cadmium, some enzymes are inactivated through direct displacement from their binding site [10]. The cellular pro-oxidative stress induced by cadmium is most likely mediated by disruption of redox homeostasis associated with mishandling of redox-active transition metals result in lipid peroxidation, membrane protein damage, DNA damage and alter gene expression [11]. Nevertheless, cadmium is not able to induce reactive oxygen species (ROS) directly because it is not effective of accepting or donating electrons under physiological conditions [12]. Even so, cadmium induces genetic damage, mutagenesis and inhibits DNA repair system [13].

A growing interest in the scientific community has been focused on plausible manners of protection from adverse effects induced by cadmium exposure. Because numerous effects to cadmium toxicity result from its genotoxic as far as pro-oxidative properties, it seems reasonable that special attention should be directed to agents that can prevent or reduce this metal-induced oxidative stress and genetic damage in several tissues, organs and systems such as liver, and peripheral blood cells [14,15]. Many authors assume that free radical scavengers and antioxidants are feasible for protecting against cadmium toxicity. Among them, fruits and vegetables have recognized nutritional value for human health mainly due to neutralization of reactive oxygen species. Of particular interest, carrot has high nutritional value as a result of bioactive constituents present in the vegetable [16].

The aim of this study was to investigate if purple carrot extract is able to protect against the noxious activities induced by cadmium exposure in multiple organs of rats. For this purpose, histopathological analysis, genotoxicity and oxidative status were investigated in this setting.

## 2. Materials and methods

### 2.1. Animals and experimental design

All experimental protocols involving animals are conformed to procedures described in the Principles for the Use of Laboratory Animals Guidelines. The study was approved by the Animal Ethics Committee of Federal University of Sao Paulo, UNIFESP, SP, Brazil (Protocol CEUA no. 4698240214).

A total of twenty Wistar rats weighing 250 g on the average, and 8 weeks age were distributed into four groups (n = 5), as follows: control group (non-treated group, CTRL); cadmium group (Cd) and purple carrot extract groups at 400 mg/L or 800 mg/L. All animals were provided from Development Center of Experimental Models for Medicine and Biology (CEDEME) of Federal University of São Paulo, SP, Brazil, and they were maintained under controlled conditions of temperature (23 ± 1 °C), light–dark periods of 12 h and free access to water and diet. The experimental design was established in previous studies conducted by our research group [17,18].

Animals from control group received a single intraperitoneal (ip) water injection while those from the groups Cd and purple carrot extract received a single ip injection of cadmium chloride (1.2 mg/kg body weight) as established elsewhere [17]. After 15 days, purple carrot groups received purple carrot extract (Hansen, Campinas Brasil) for 400 mg/L or 800 mg/L in drinking water ad libitum. The daily dose was calculated in order to provide the equivalent in humans of 2 g polyphenols/day taking into consideration the rat metabolism (twice faster than humans). The amount was reported by the American Dietetic Association (ADA) as sufficient to promote beneficial health effects [19]. This value corresponds to 400 mg/L of purple carrot extract [19]. Control and cadmium groups received drinking water during the same experimental period ad libitum. All animals were checked daily for behavior and general health conditions and body mass was recorded weekly. At the end of the experimental period, all animals were anesthetized with inhalational anesthetic halothane (Tanohalo™, Cristália™, SP, Brazil) and euthanazied for tissue collection.

### 2.2. Quantification of total phenols, determination of radical scavenging activity and anthocyanins

Total phenols were measured by the Folin–Ciocalteu assay using gallic acid (Sigma–Aldrich®, St. Louis, MO, USA) for the standard curve and the results being expressed in mg gallic acid equivalents (GAE)/kg. The readings (in triplicates) were taken at 740 nm using a Genesis 2 spectrometer. For the evaluation of the antioxidant activity in vitro, the DPPH (1,1-diphenyl-2-picrylhydrazil) (Sigma–Aldrich®, Steinheim, BW, Germany) assay was used based on the methods of Brand-Williams [20]. The absorbance was measured with a Beckman spectrometer at 517 nm before addition of samples and after 30 min; the difference was plotted on a vitamin C (ascorbic acid) (Merck®, USA) standard curve. Analyses were carried out in triplicates and the results expressed in milligrams Vitamin C equivalents (VCEAC)/kg. The presence of anthocyanins was measured in duplicate by UV–vis spectroscopy as described elsewhere [21].

### 2.3. Characterisation of purple carrot extract by electrospray ionization mass spectrometer (ESI-MS)

The sample (100 mg) was extracted with 1000 μL of methanol. The extract solution was thoroughly mixed for 1 h with magnetic bar and filtrated. Then 80 μL of this extract was diluted in 1000 μL of methanol. Formic acid (0.1%) was added to evaluate samples in the positive ion mode and ammonium hydroxide (0.1%) was added to evaluate samples in the negative ion mode. Samples were direct infused and analyzed by a 7.2T LTQ FT (FT-ICR) Ultra mass spectrometer (ThermoScientific, Bremen, Germany). The full scan spectra were acquired in the range of $m/z$ 100–1200. General ESI conditions were as follow: gas pressure of 0.3 psi, capillary voltage of ±3.0 kV, tube lens of +115 and −125 V, capilar temperature of 280 °C and a flow rate of 5 $\mu L\, min^{-1}$. Mass spectra were processed via the Xcalibur 2.0 software (ThermoScientific, Bremen, Germany).

### 2.4. Histopathological analysis

Histopathological changes in liver, including steatosis and inflammation, were analyzed by the semi-quantitative method (Table 1) according to Aguiar et al. [22].

### 2.5. Single cell gel (Comet) assay

The protocol used for peripheral blood and liver cells followed the guidelines outlined by Tice et al. [23]. Peripheral blood was collected by cardiac puncture and liver cells were obtained by liver tissue maceration with PBS. Cells were transferred to individual plastic tubes, containing 1 mL of cold phosphate buffer solution (PBS, $Ca^{2+}$, $Mg^{2+}$ free, pH 7.3), and centrifuged for 5 min, 1000 rpm, at room temperature. The supernatant was removed and the cell suspensions (∼10 μL) were used for single cell gel (Comet) assay. A volume of 10 μL of cellular suspension was added to 120 μL of 0.5% low-melting point agarose at 37 °C, layered onto a pre-coated slide with 1.5% regular agarose, and covered with a coverslip. After brief agarose solidification in refrigerator, the coverslip was removed and the slides immersed in

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