Pathophysiology 26 (2019) 263–269

Contents lists available at ScienceDirect

Pathophysiology

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

The protective effect of grape skin or purple carrot extracts against cadmium intoxication in kidney of rats

Samuel Rangel Claudio[a], Flavia Andressa Pidone Ribeiro[b], Eliene Cezario De Lima[a], Aline Boveto Santamarina[b], Luciana Pellegrini Pisani[b], Camilo Seabra Dias Pereira[c], Celina Tizuko Fujiyama Oshima[a], Daniel Araki Ribeiro[b,*]

[a] Department of Pathology, Federal University of Sao Paulo, UNIFESP, SP, Brazil
[b] Department of Biosciences, Federal University of Sao Paulo, UNIFESP, SP, Brazil
[c] Department of Sea Sciences, Federal University of Sao Paulo, UNIFESP, SP, Brazil

ARTICLE INFO

Article history:
Received 27 March 2019
Received in revised form 15 June 2019
Accepted 27 July 2019

Keywords:
Cadmium
Antioxidants
Kidney
Rats

ABSTRACT

The aim of this study was to evaluate the protective effect of grape skin or purple carrot extracts against cadmium-induced intoxication in rats' kidneys. For this purpose, 30 male Wistar rats were distributed into six groups (n = 5), as follows: control group; cadmium group and groups treated with grape skin at 175 or 350 mg / L doses; or purple carrot extract at 400 mg / L or 800 mg / L doses, by drinking water. In the group exposed to cadmium, histopathological analysis revealed severe tissue injury as a result of coagulation necrosis, congested vessels and inflammatory infiltrate. Animals treated with grape skin or purple carrot extracts improved the histopathological changes induced by cadmium. 8-OHdG immunoexpression and catalase gene expression decreased in rats treated with purple carrot or grape skin extracts. Grape skin extract was able to increase SOD-CuZn gene expression as well. Toll-like signaling pathway (TLR2, PIKK and TRAF6) and cytochrome c expressions were not altered after the treatment with grape skin or purple carrot extracts. Taken together, we conclude that grape skin and purple carrot extracts had a protective effect on the rats' kidneys after cadmium intoxication, by means of tissue regenerating tissue regeneration and antioxidant properties, grape skin extract being more effective for this purpose.

1. Introduction

Environmental exposure to cadmium has been widely reported in scientific literature because of its high bioaccumulation; it can remain in the human body for two to three decades. The non-essential metal comes from a variety of sources such as air, soil, food, some occupations and especially cigarettes, which have a considerable amount of the metal in their composition [1–3].

Human exposure to cadmium can lead to systemic injury of many organs such as kidneys, lungs, liver, reproductive system and bones. The main biological action is due to the exponential increase of reactive oxygen species, damaging tissues and/or cells. Cadmium is classified by the International Agency for Research on Cancer (IARC) as a carcinogen for humans and experimental animals [4,5].

It has been well established that the kidney is one the most important organs for cadmium-induced intoxication, causing polyuria and proteinuria. Many studies have been conducted on the biological actions promoted by cadmium, in order to identify the molecular interaction between non-essential metals and renal parenchyma [6]. Unfortunately, this has not yet been fully clarified.

Natural products represent a source of innovative therapeutic alternatives for treating several diseases [7,8]. Among bioactive substances available from plant origin, polyphenols are one of the most investigated because their beneficial affects on health. In fact, epidemiological, clinical and in vitro studies have shown multiple positive biological effects closely related to polyphenols present in the diet, having antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic and antiviral activities [9–11].

In recent years, interest has been focused on the production and extraction of polyphenols from various foods, such as the purple carrot (*Daucus carota*). The extract is used as a natural dye in the food industry. Besides the use as a natural feed additive, the consumption of purple carrots has increased in Western Europe, due to the relevant contribution of polyphenols incorporated into the regular diet [12].

The protective effects attributed to polyphenols from grapes are well established in scientific literature [13–15]. Particularly, our

* Corresponding author at: Department of Biosciences, Federal University of Sao Paulo, UNIFESP, Av. Ana Costa, 95, Vila Mathias, Santos, SP, 11060-001, Brazil.
E-mail address: daribeiro@unifesp.br (D.A. Ribeiro).

https://doi.org/10.1016/j.pathophys.2019.07.001
0928-4680/

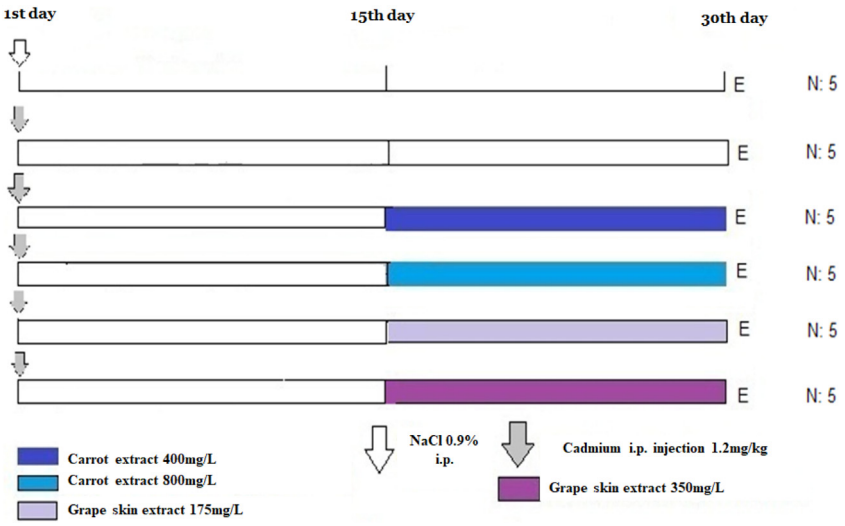

Fig. 1. Experimental design of this study.

research group has investigated the biological action of grape skin and purple carrot extracts in murine models following cadmium intoxication, with significant results for reducing oxidative damage on hepatic and blood cells [16,17]. We were also able to evaluate the chemopreventive activity of purple carrot extract in rat tongue carcinogenesis induced by 4-nitroquinoline 1-oxide (4NQO) [18]. The results revealed that purple carrot extract protected the oral mucosa against the chemical carcinogen 4NQO by means of anti-mutagenic, anti-proliferative and anti-inflammatory actions [18]. Thus, it would be interesting to know if, and to what extent, the bioactive substances present in purple carrot and grape skin are able to protect kidney cells against cadmium-induced intoxication. This would provide identification of tissues and / or organs which are more sensitive to cadmium intoxication and of the role of grape or purple carrot extracts in rats' kidneys.

The aim of this study was to investigate whether grape skin and purple carrot extracts may protect rats' kidneys against the harmful activities induced by exposure to cadmium. For this purpose, histopathological analysis, genotoxicity, anti-oxidant and inflammatory activities were evaluated.

2. Material and methods

2.1. Animals and experimental design

All experimental protocols involving animals were conducted as described in the Principles for the Use of Guidelines for Laboratory Animals. The study was approved by the Animal Ethics Committee of the Federal University of São Paulo, UNIFESP, São Paulo, Brazil (CEUA, protocol number 6615170117).

All animals were purchased from the Center for the Development of Experimental Models of Medicine and Biology (CEDEME) at Federal University of São Paulo, São Paulo, and were kept under controlled conditions of temperature (23 ± 1 °C), light- dark of 12 h and free access to water and diet. A total of 30 male Wistar rats weighing 250 g on average and 8 weeks of age were distributed into six groups (n = 5), as follows: control group (untreated group) cadmium group (Cd), and groups of grape extracts at 175 or 350 mg / L doses and groups of purple carrot extracts at 400 mg / L or 800 mg / L doses. The daily dose was calculated to be equivalent to humans, i.e, 2 g of polyphenols / day taking into account the murine metabolism (two times faster than humans). The total amount of polyphenols has sufficient capacity to promote beneficial effects on human health as described elsewhere [19]. These values were 175 mg / l of grape extract or 400 mg / L of purple carrot extract. The experimental design is illustrated in Fig. 1.

Animals from control group received a single intraperitoneal (i.p.) of water injection. Cadmiun and purple carrot and cadmium and grape skin extract groups received a single i.p. injection of cadmium chloride (1.2 mg / kg body weight). After 15 days, the experimental groups were treated with the respective extracts at the doses previously adopted in this study for 15 days in drinking water. The experimental design is described elsewhere [20,21].

Chemical characterization of grape and carrot extracts was previously performed by our research group in order to identify the main bioactive substances. These data were published in the scientific literature [16,17]. For purple carrot extract, the following bioactive substances were identified: cyanidin-3-(2″-xylosyl-galactoside), cyanidin-3-(2″xylopiranose-6″-(6‴-feruloylglucopyranose)-galactopyranose), cyanidin-3-(2″xylosyl-6″-(6‴-sinapoylglucoside)-galactoside), caffeoyl N-tryptophan, caffeoyl N-tryptophan hexoside, caffeoylquinic acid and citric acid. The following bioactive substances were recognized by ESI mass spectrometry in the grape skin extract: Peonidin, Malvidin, Peonidin-3-O-Monoglucoside, Petunidin-3-O-Monoglicoside, Malvidin-3-O-Monoglicoside, Peonidin-3-acetylglucose, Petunidin-3-O-(6-O-Acetyl)Monoglicoside, Malvidin-3-O-(6-O-Acetyl)Monoglicoside, Peonidin-3-O-Rutinoside, Malvidin-3-O-("6-p-coumaryl-glucoside), and Malvidin-caffeoyl-3-glucoside.

2.2. Histopathological analysis

Histopathological evaluation was made to the slides stained with hematoxylin and eosin (H.E.) by light conventional microscope (Olympus, Optical Co. Ltd, Tokyo, Japan). Histopathological changes were evaluated by semi-quantitative scoring method: no injury (0), mild: <25% (1), moderate: <50% (2), severe: <75% (3) and very severe: >75% (da Silva et al. 2014). The following changes were considered: tissue coagulation necrosis, tubular lesions and glomerular destruction, hemorrhagic areas and chronic inflammatory infiltrate.

2.3. Immunohistochemistry for 8OHdG

The immunohistochemistry for 8OHDG was performed as described by Ruiz et al. (2018). For this purpose, it was used the

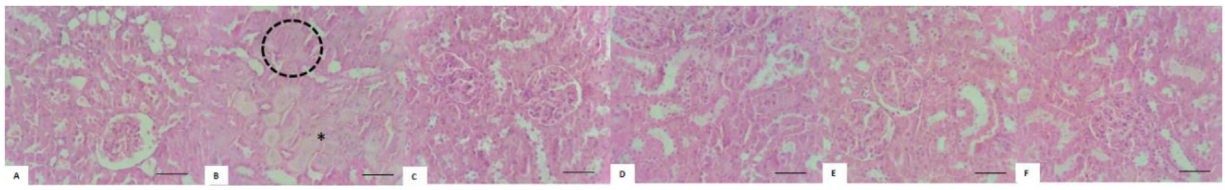

Fig. 2. Photomicrographies of renal tissue in animals intoxicated by cadmium and treated with extracts of purple carrot and grape skin. A-control; B-cadmium; C-cadmium and purple carrot extract at 175 mg; D- cadmium and purple carrot extract at 350 mg; E- cadmium and grape skin extract at 400 mg and F- cadmium and grape skin extract at 800 mg. Note the presence of extensive coagularion necrosis (asterisk) followed by glomerule destruction (circle) in cadmium group. H.E. staining. Bar = 32 um.

anti-8-hydroxy-20-deoxyguanosine (8OHDG, Santa Cruz Biotechnologies Inc™, MO, USA) at 1:100 dilution. The analysis was based on scores, taking into account the presence or absence of immunopositive cells as well as the extent of the stained sections, as follows: no staining (0), weak staining (1), moderate staning (2) and strong staning (3) [16].

2.4. Single cell gel (comet) assay

The single cell gel (comet) assay followed the guidelines described by Tice et al. [22]. A total of 25 visible comets per animal (25 cells from each slide) were examined blindly by one observer at x400 magnification using a fluorescent microscope connected to an image analysis system (Comet Assay II, Perceptive Instruments™, Suffolk, Haverhill, UK). To measure DNA damage, tail moment was adopted in this study. The parameter is defined as the product of the tail length and the fraction of DNA in the comet tail was used [22].

2.5. Real time PCR (qPCR)

Total RNA was isolated from kidney using cold Trizol Reagent (Invitrogen™, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA samples were treated with DNAse (DNase Amplification Grade™, Applied Biosystems™, Foster City, CA, USA). cDNA synthesis was made using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, Foster City, CA, USA) according to the manufacturer's instructions. Real-time PCR was performed using a 7500 Fast Real- Time PCR System (Applied Biosystems™, Foster City, CA, USA) and the Power SYBR™Green Kit (PCR Master Mix 2×, Applied Biosystems™, Foster City, CA, USA). The following primers sequence were used in this study: GAPDH: sense 5′-CAA CTC CCT CAA GAT TGT CAG CAA-3′ and anti-sense 5′-GGC ATG GAC TGT GGT CAT GA-3′, SOD-CuZn: sense 5′-CCA GTG CAG GAC CTC ATT TT-3′ and anti-sense 5′-CCT TTC CAG CAG TCA CAT TG-3′, SOD-Mn: sense 5′-AAC ATT AAC GCG CAG ATC A-3′ and anti-sense 5′-AATATG TCC CCC ACC ATT GA-3′, Catalase: sense 5′-AGC GGA TTC CTG AGA GAG TG-3′ and anti-sense 5′-GAG AAT CGA ACG GCA ATA GG-3′, Cytochrome C: sense 5′- AGA ATG TTG GCT ACC AGG GC-3′ and anti-sense 5′-GGA TGG GGC CAT ACA CGT AG-3′. PCR reactions were conduced in duplicate for 40 cycles of 95 °C for 10 min, 95 °C for 15 s and 60 °C for 1 min. An amplification efficiency curve using different cDNA dilutions was performed for each gene tested.

2.6. Western blotting analysis

The western blotting was performed according to the described by Soares et al. [18]. The following primary antibodies were used: TLR2 (sc-101744) purchased from Santa Cruz Biotechnology, Inc.(Santa Cruz, CA, USA). The IKK (ab2064), TRAF6 (ab33915) and GADPH (ab6276), which were purchased to ABCAM (Cambridge, UK). The intensities of each band sample were quantified by Image J software (Image J, National Institute of Health, Maryland, USA). While performing calculations of each band obtained for analysis of proteins of interest for this study was normalized using GADPH levels of the respective membrane.

Table 1
Histopathological changes in kidney of rats intoxicated by cadmium and treated with purple carrot and grape skin extracts.

ESCORE	0	1	2	3	4
CTRL	**5**	0	0	0	0
Cd	0	0	0	**5**	**0**
Purple **carrot. 400 + (Cd)** *	0	**4**	**1**	0	0
Purple **Carrot 800 + (Cd)** *	0	**4**	**1**	0	0
Grape skin 175 + (Cd)*	0	**4**	**1**	0	0
Grape skin 350 + (Cd)*	0	**4**	**1**	0	0

* $p < 0.05$ compared to the cadmium (Cd) group. CTRL: control group; N = 5 per group.

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (SD). Real Time PCR and single cell gel (comet) assay data were evaluated by two way analysis of variance (two –way ANOVA) followed by Tukey's multiple comparisons test. Histopathology, immunohistochemistry and western blotting analyzes were evaluated by Kruskal-wallis non-parametric tested followed by Dunn's test. $p < 0.05$ was considered to be significant.

3. Results

3.1. Histopathological analysis

In the control group, the histopathological analysis revealed cortex with centralized nuclei, glomeruli and preserved tubules, demonstrating ordinary architecture (Fig. 2A). In animals exposed to cadmium, an extensive tissue coagulation necrosis, tubular lesions and glomerular destruction, hemorrhagic areas and chronic inflammatory infiltrate were noticed (Fig. 2B). The animals exposed to cadmium and then treated with purple carrot extract at doses of 400 and 800 mg/L and grape skin extract at doses of 175 and 350 mg/L, restored the tissue damage caused by the non-essential metal, as demonstrated by normal morphological characteristics (Figs. 2C and 2D). Numerical data showed significant statistical differences ($p < 0.05$) for all treated groups when compared to the group exposed to cadmium but not treated. In animals treated with grape skin extract at doses of 175 and 350 mg/L, a significant reduction of tissue damage was detected (Figs. 1E and 2F). Statistically significant differences ($p < 0.05$) were detected in both these groups. The numerical results are presented in Table 1.

3.2. Immunohistochemistry for 8-OHdG

Immunoexpression of 8-OHdG was detected in the cytoplasm and nucleus of kidney cells. In the control group, a low expression of 8-OHdG was detected, mainly in the renal tubules (Fig. 3A). In the cadmium-intoxicated group, a high expression of 8-OHdG was detected in the renal parenchyma (Fig. 3B). In the groups treated

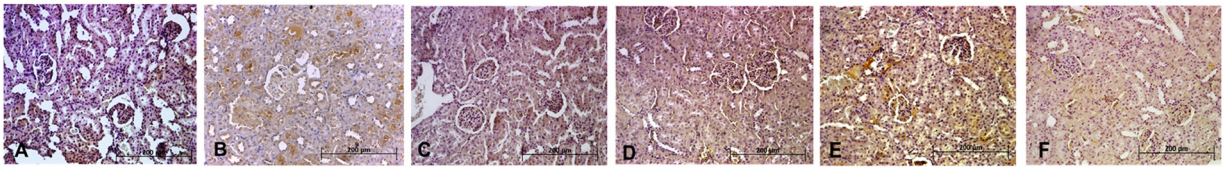

Fig. 3. Immunohistochemistry for 8-OHDG in renal tissue of rats exposed to cadmium and treated with purple carrot and grape skin extracts. A-control; B-cadmium; C-cadmium and purple carrot extract at 175 mg; D- cadmium and purple carrot extract at 350 mg; E- cadmium and grape skin extract at 400 mg and F- cadmium and grape skin extract at 800 mg. Immunohistochemistry. Avidin-Biotin-Peroxidase System. Bar = 36 um.

Table 2
Immunohistochemical analysis of 8-OHdG in renal tissue of animals exposed to cadmium and treated with extracts of grape skin and purple carrot.

ESCORE	0	1	2	3	4
CTRL	0	4	1	0	0
Cd	0	0	2	**3**	**0**
Purple **carrot** 400**+ (Cd)** *	3	**1**	**1**	0	0
Purple **carrot 800 + (Cd)** *	3	**1**	**1**	0	0
Grape skin 175 + (Cd)*	3	**1**	**1**	0	0
Grape skin 375 + (Cd)*	4	**1**	**0**	0	0

* $p < 0.05$ compared to the cadmium (Cd) group. CTRL: control group, N = 5 per group.

with grape skin and purple carrot extracts, a reduction in expression of 8-OHdG was noticed, when compared to the group exposed to cadmium but otherwise untreated (Figs. 3C and 3F).

Semi-quantitative analysis showed a significant ($p < 0.05$) decrease in 8-OHdG immunoexpression from animals treated with purple carrot extract or grape skin extract for the two doses tested in each group. These results are shown in Table 2.

3.3. Single cell gel (comet) assay

The results of the single cell gel (comet) assay are presented in Fig. 4. Cadmium group showed a significant increase for DNA damage ($p < 0.05$) when compared to the control group. Therefore, it appears that cadmium is a potent genotoxic agent for kidney cells. In the groups treated with purple carrot extract at 400 mg or 800 mg doses, no significant differences was observed ($p > 0.05$). However, grape skin at extract at 350 mg promoted a significant reduction in the DNA strand breaks, whereas the low dose of grape skin extract (175 mg) was unable to significantly reduce genetic damage when compared to the cadmium group ($p > 0.05$). There was evidence of a significant reduction of the tail moment values in animals treated with grape skin extract at 350 mg dose, demonstrating an anti-genotoxic effect in the kidney cells.

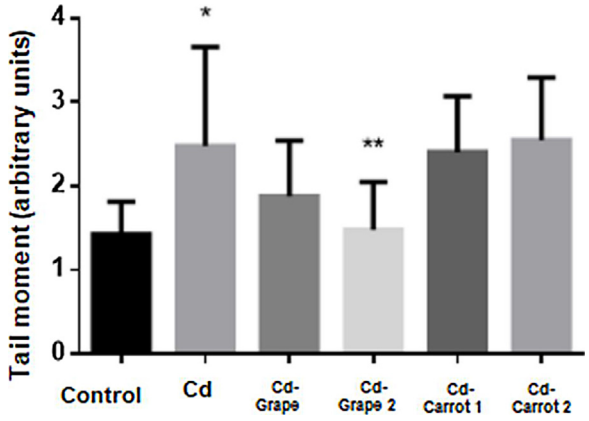

Fig. 4. DNA damage (expressed as tail moment) in the kidney of rats exposed to cadmium and treated with purple carrot extract or grape skin extracts. * $p < 0.05$ when compared to the control group (Cd); ** $p < 0.05$ when compared to the cadmium group (Ctrl).: Grape 1: 175 mg; Grape 2: 350 mg; Carrot 1; 400 mg; Carrot 2: 800 mg.

3.4. Real time PCR

Cadmium was able to reduce the expression of SOD-CuZn ($p < 0.05$) in kidney cells. In animals treated with grape skin extract at 175 mg or 350 mg, significant statistical differences ($p < 0.05$) in SOD-CuZn expression were observed when compared to the cadmium group. However, there were no significant statistical differences ($p > 0.05$) in the groups treated with purple carrot extract for both doses tested. In a similar way, the expression of SOD-Mn did not present remarkable changes for both extracts investigated in this study.

Catalase expression showed a significant increase ($p < 0.05$) in the animals exposed to cadmium, when compared to the control group. A reduced expression was also observed for all groups treated with purple carrot and grape skin extracts, presenting significant statistical differences ($p < 0.05$) when compared to animals only exposed to cadmium. These results are presented in Fig. 5.

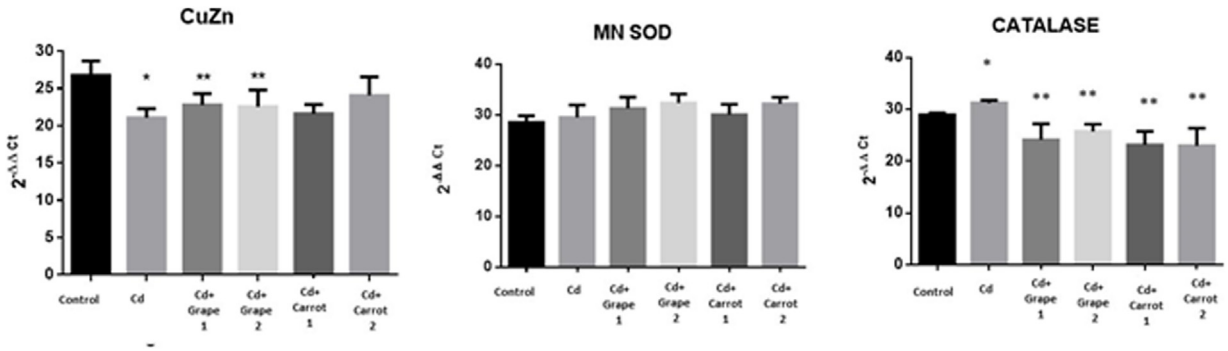

Fig. 5. Expression of SOD-CuZn, SOD Mn and catalase in the kidneys of rats exposed to cadmium and treated with purple carrot and grape extracts. * $p < 0.05$ when compared to the cadmium group. ** $p < 0.05$ when compared to the Cadmium group. CTRL: Grape 1: 175 mg; Grape 2: 350 mg; Carrot 1; 400 mg; Carrot 2: 800 mg.

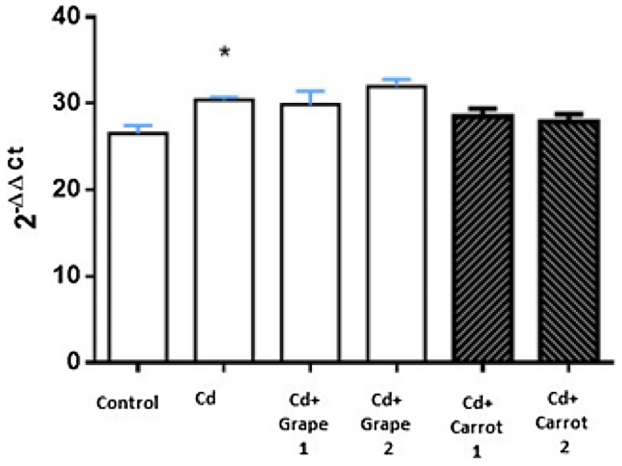

Fig. 6. Expression of cytochrome c in the kidneys of rats exposed to cadmium and treated with purple carrot and grape extracts. * $p < 0.05$ when compared to the cadmium group. CTRL: Grape 1: 175 mg; Grape 2: 350 mg; Carrot 1; 400 mg; Carrot 2: 800 mg.

Cadmium increased cytochrome c expression in kidney cells. Statistically significant differences ($p < 0.05$) were detected between cadmium and control groups. Conversely, treatments with grape skin and purple carrot extracts did not interfere with the expression of cytochrome c, i.e., no statistically significant differences ($p > 0.05$) were detected. The results are shown in Fig. 6.

3.5. Western blotting

Cadmium increased TLR2 protein expression ($p < 0.05$) when compared to control group. Nevertheless, not all treatments investigated in this study were able to modulate the expression of TLR2 and pIKKα. Similarly, the expression of TRAF6 was not altered in animals exposed to cadmium nor those treated with purple carrot and grape skin extracts. These results are shown in Fig. 7.

4. Discussion

The aim of this study was to evaluate the nutraceutical potential of grape skin and purple carrot extracts following cadmium intoxication of rats' kidneys. For this purpose, histopathological evaluation, immunohistochemical analysis for 8-OHdG, genotoxicity, antioxidant activity and toll-like signaling pathway were used to elucidate the putative mechanisms of action induced by these bioactive substances. To our knowledge, this approach has never before been documented in scientific literature (Fig. 7).

It is well established that the kidney plays a crucial role following cadmium exposure [23]. In fact, cadmium is able to induce severe lesions in the renal parenchyma, as evidenced by coagulation necrosis, vascular congestion and inflammatory process [24]. When the animals were treated with purple carrot or grape skin extracts, there was a significant improvement in renal morphology, for both doses in this setting. Therefore, it is plausible that extracts rich in bioactive substances detected by chemical analysis, such as cyanidin, petunidin, caffeoyl, peonidin and malvidin, etc., induced tissue regeneration after cadmium intoxication. All animals treated with both extracts showed an absence of areas of coagulation necrosis, with few foci of inflammatory infiltrate and vascular congestion. Surprisingly, the morphological evaluation for studying the nutraceutical potential of bioactive compounds against cadmium-induced renal toxicity is scarce in existing literature [25]. Recent data and studies on this subject investigated antioxidant system, inflammatory biomarkers and renal function [26,27]. No studies have yet been conducted into the effects of using purple carrot extract. This clearly demonstrates the need to investigate morphological characteristics induced by these bioactive substances following exposure to non-essential metals, such as cadmium. Certainly, this analysis will contribute to scientific literature.

To further elucidate the cell signaling pathways that are closely involved in the regeneration of the renal tissue, this study evaluated genetic damage and antioxidant activity in the kidney by means of comet assay and immunohistochemistry, respectively. Firstly, our results demonstrated that purple carrot and grape skin extracts were able to decrease oxidative stress by decreasing the 8-OHdG

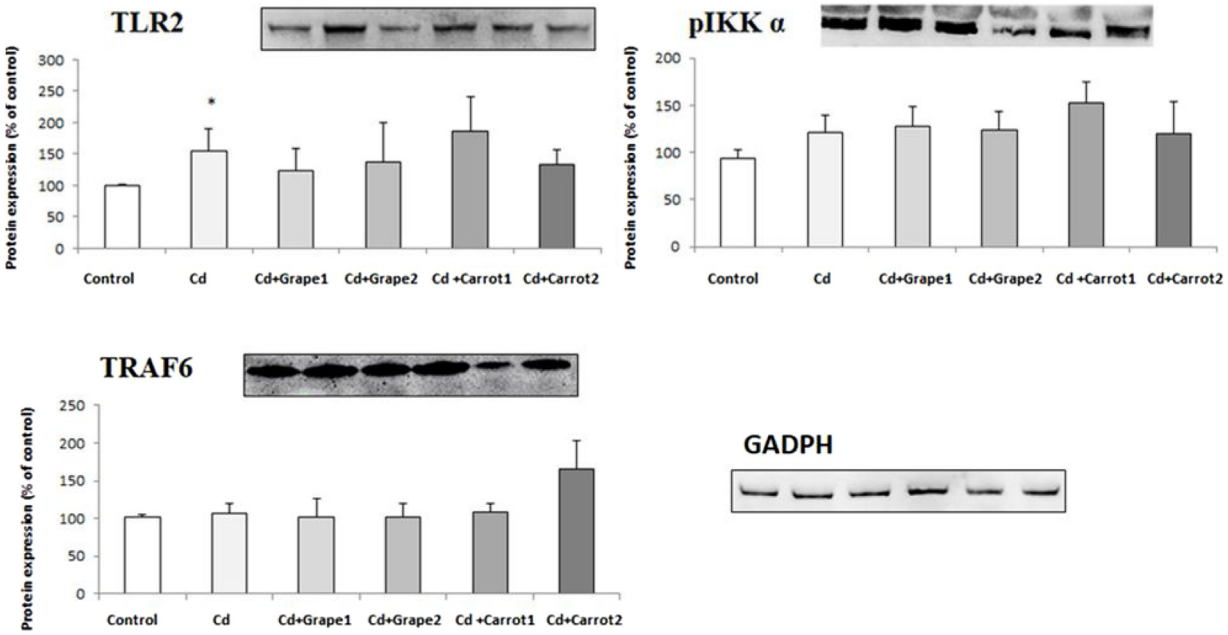

Fig. 7. Western blotting analysis of TLR-2, pIKKα, and TRAF6 proteins in animals exposed to cadmium and treated with grape skin and purple carrot extracts. * $p < 0.05$ when compared to the control group. Grape 1: 175 mg; Grape 2: 350 mg; Carrot 1; 400 mg; Carrot 2: 800 mg.

immunoexpression in kidney cells. This was not supported by genotoxicity data, since only the highest dose of grape skin extract promoted an antigenotoxic effect. In an earlier study conducted by Chomchan et al. [28], the authors demonstrated that selenium was able to exert an antigenotoxic effect on renal cells after exposure to cadmium. Using non-mammalian species, other authors have also evidenced the genotoxic action induced by cadmium as well as the antigenotoxic effect of bioactive compounds following cadmium exposure [29,30]. To date, no study has been able to evaluate 8-OHdG immunoexpression in rats' kidneys exposed to cadmium associated with bioactive compounds, such as purple carrot or grape skin extracts. Taken as a whole, we conclude that cadmium induces oxidative stress in kidney cells. Certainly, this activity is one of the underlying mechanisms responsible for tissue injury, as already mentioned by others [31]. Grape skin and purple carrot extracts exert some antioxidant activity on genetic material against oxidative stress induced by cadmium.

Regarding antioxidant activity, our results demonstrated that grape skin extract was able to increase SOD-CuZn expression in animals previously intoxicated with cadmium. On the other hand, catalase gene expression decreased for all groups treated with grape skin or purple carrot extracts, for both doses used. No statistically significant differences were noticed to SOD-Mn expression between groups. It is important to stress that some studies have revealed the modulating activity of Tinospora cordifolia stem extract, zinc, selenium and Solanum torvum Swartz following cadmium exposure [32,33]. Our results are in agreement with these aforementioned studies. Nevertheless, grape skin extract exerts a more pronounced effect on the expression of the antioxidant enzymes SOD-CuZn and catalase whereas the purple carrot extract acted on only catalase gene expression. Certainly, the bioactive substances present in the grape skin extract are more powerful for modulating the enzymatic anti-oxidant system when compared to the purple carrot extract.

Recently, it has been established that mitochondria play a key role following cadmium intoxication [34]. This is because the oxidative stress induced by cadmium in kidney cells promotes disruption of this organelle, causing cellular death. Cytochrome c is a protein located on the inner surface of the mitochondrial membrane, which participates in the cellular respiration and cell death by apoptosis. Recent studies have demonstrated that cadmium is able to activate apoptosis in kidneys [35], but few studies have investigated the role of cytochrome c in the context of cadmium and bioactive extracts. Our results demonstrated that cytochrome c is differentially expressed in animals exposed to cadmium. Some authors have demonstrated that cytochrome c expression is induced by cadmium and selenium and/or puerarim, both in vivo and in vitro [36,37]. Nevertheless, grape skin and purple carrot extracts were not able to alter the cytochrome c expression in kidney cells.

Toll-like signaling pathway is a complex cascade composed of TLR, IKK, TRAF6 and many other proteins. It actively participates in several biological actions, such as the inflammatory response in eukaryotic cells. A previous literature review published by our research group established the underlying mechanisms of bioactive compounds and toll-like signaling pathway for promoting tissue homeostasis [38]. In this study, the results demonstrated that exposure to cadmium caused an increased in the expression of TLR2 in rats. These results are consistent with previous studies using several test systems [39,40]. However, the biological action of the extracts obtained from purple carrot and grape skin did not interfere with the expression of proteins from the toll-like signaling pathway. Further studies are needed to elucidate the issue.

In conclusion, purple carrot and grape skin extracts promote tissue regeneration against cadmium toxicity by means of antioxidant action in rats' kidneys. It appears that grape skin extract is more effective compared to purple carrot extract.

Declaration of Competing Interest

None declared.

Acknowledgments

This work was supported by CAPES (Coordination for the Improvement of Higher Education Personnel) and CNPq (The Brazilian National Council for Scientific and Technological Development).

References

[1] S. Satarug, S.H. Garrett, M.A. Sens, D.A. Sens, Cadmium, environmental exposure and health outcomes, Environ. Health Perspect. 118 (2) (2010) 182–190.
[2] A. Engström, K. Michaëlsson, Y. Suwazono, A. Wolk, M. Vahter, A. Akesson, Long-term cadmium exposure and the association with bone mineral density and fractures in a population-based study among women, J. Bone Miner. Res. 26 (3) (2011) 486–495.
[3] M. Rafati Rahimzadeh, M. Rafati Rahimzadeh, S. Kazemi, A.A. Moghadamnia, Cadmium toxicity and treatment, an update, Caspian J. Intern. Med. 8 (3) (2017) 135–145.
[4] W. Donpunha, U. Kukongviriyapan, K. Sompamit, P. Pakdeechote, V. Kukongviriyapan, P. Pannangpetch, Protective effect of ascorbic acid on cadmium-induced hypertension and vascular dysfunction in mice, Biometals 24 (1) (2011) 105–115.
[5] International agency for research on cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer, 1994.
[6] F. Thévenod, N.A. Wolff, Iron transport in the kidney, implications for physiology and cadmium nephrotoxicity, Metallomics 8 (1) (2016) 17–42.
[7] O.J.D. Cruz, F.M. Uckun, Clinical development of microbicides for the prevention of HIV infection, Curr. Pharm. Res. 10 (2004) 315–336.
[8] M. Helfer, H. Koppensteiner, M. Schneider, S. Rebensburg, S. Forcisi, C. Muller, P. Schmitt-Kopplin, M. Schindler, R. Brack-Werner, The root extract of the medicinal plant Pelargonium sidoides is a potent HIV-1 attachment inhibitor, PLoS One 9 (1) (2014), e87487.
[9] J. Gusman, H. Malonne, G. Atassi, A repraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol, Carcinogenesis 22 (8) (2001) 1111–1117.
[10] A.P.B. Gollücke, O. Aguiar Jr, L.F. Barbisan, D.A. Ribeiro, Use of polyphenols against carcinogenesis, putative moléculas mechanisms of action using in vitro and in vivo test systems, J. Med. Food 16 (3) (2013) 1–7.
[11] S. Pasetto, V. Pardi, R.M. Murata, Anti-HIV-1 activity of flavonoid myricetin on HIV-1 infection in a dual-chamber in vitro model, PLoS One 9 (12) (2014), e115323.
[12] R. Singh, N. Gautam, A. Mishra, R. Gupta, Heavy metals and living systems, an overview, Indian J. Pharmacol. 43 (3) (2011) 246–253.
[13] A.P.R. Paiotti, R. Artigiani Neto, P. Marchi, R.M. Silva, V.L. Pazine, J. Noguti, M.M. Pastrelo, A.P.B. Gollücke, S.J. Miszputen, D.A. Ribeiro, The anti-inflammatory potential of phenolic compounds in grape juice concentrate (G8000) on 2,4,6-trinitrobenzene sulphonic acid-induced colitis, Br. J. Nutr. 110 (2013) 973–980.
[14] J. Yang, Y.Y. Xiao, Grape phytochemicals and associated health benefits, Crit. Rev. Food Sci. Nutr. 53 (2013) 1202–1225.
[15] A. Teixeira, N. Baenas, R. Dominguez-Perles, Natural bioactive compounds from winery by-products as health promoters, a review, Int. J. Mol. Sci. 15 (2014) 15638–15678.
[16] S.R. Claudio, A.P. Gollucke, H. Yamamura, D.R. Morais, G.A. Bataglion, M.N. Eberlin, R.C. Peres, C.T. Oshima, D.A. Ribeiro, Purple carrot extract protects against cadmium intoxication in multiple organs of rats, genotoxicity, oxidative stress and tissue morphology analyses, J. Trace Elem. Med. Biol. 33 (2016) 37–47.
[17] A.P. Boiago Gollucke, S.R. Claudio, H. Yamamura, D.R. Morais, G.A. Bataglion, M.N. Eberlin, O. Aguiar Jr, D.A. Ribeiro, Grape skin extract mitigates tissue degeneration, genotoxicity, and oxidative status in multiple organs of rats exposed to cadmium, Eur. J. Cancer Prev. 227 (1) (2018) 70–81.
[18] G.R. Soares, C.F.G. de Moura, M.J.D. Silva, W. Vilegas, A.B. Santamarina, L.P. Pisani, D. Estadella, D.A. Ribeiro, Protective effects of purple carrot extract (Daucus carota) against rat tongue carcinogenesis induced by 4-nitroquinoline 1-oxide, Med. Oncol. 35 (4) (2018) 54.
[19] C.M. Hasler, A.C. Brown, American dietetic association. Position of the American Dietetic Association, functional foods, J. Am. Diet. Assoc. 109 (4) (2009) 735–746.
[20] C.F. De Moura, F.A.P. Ribeiro, G.P. de Jesus, V.H.P. da Silva, C.T.F. Oshima, A.P.B. Golucke, O. Aguiar Jr, D.A.R. Ribeiro, Antimutagenic and antigenotoxic potential of grape juice concentrate in blood and liver of rats exposed to cadmium, Environ. Sci. Pollut. Res. 21 (2014) 13118–13126.
[21] M.J. Silva, W. Vilegas, M.A. da Silva, C.F. de Moura, F.A. Ribeiro, V.H. da Silva, D.A. Ribeiro, Mimosa (Mimosa caesalpiniifolia) prevents oxidative DNA damage induced by cadmium exposure in Wistar rats, Toxicol. Mech. Methods 24 (8) (2014) 567–574.

[22] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (3) (2000) 206–221.
[23] J. Adeline, A. Josiane, H.F. Isabelle, H.P. Florence, C. Karine, L. Marine, L. Frédéric, O. Fayçal, F. Eric, M. Jean-Marc, D. Christine, Impact of chronic and low cadmium exposure of rats, sex specific disruption of glucose metabolism, Chemosphere 207 (2018) 764–773.
[24] S. Yildirim, F.C. Celikezen, G. Oto, E. Sengul, M. Bulduk, M. Tasdemir, D. Ali Cinar, An investigation of protective effects of litium borate on blood and histopathological parameters in acute cadmium-induced rats, Biol. Trace Elem. Res. 182 (2) (2018) 287–294.
[25] S. Chen, G. Liu, M. Long, H. Zou, H. Cui, Alpha lipoic acid attenuates cadmium-induced nephrotoxicity via the mitochondrial apoptotic pathways in rat, J. Inorg. Biochem. 184 (2018) 19–26.
[26] B. Nazima, V. Manoharan, S. Miltonprabu, Grape seed proanthocyanidins ameliorates cadmium-induced renal injury and oxidative stress in experimental rats through the up-regulation of nuclear related factor 2 and antioxidant responsive elements, Biochem. Cell Biol. 93 (3) (2015) 210–226.
[27] K.A. Rho, M.K. Kim, Effects of different grape formulations on antioxidative capacity, lipid peroxidation and oxidative DNA damage in aged rats, J. Nutr. Sci. Vitaminol. (Tokyo) 52 (1) (2006) 33–46.
[28] R. Chomchan, S. Siripongvutikorn, P. Maliyam, B. Saibandith, P. Puttarak, Protective effect of selenium-enriched ricegrass juice against cadmium-induced toxicity and DNA damage in HEK293 kidney cells, Foods 7 (6) (2018) E81, pii.
[29] M. Fatima, N. Usmani, F. Firdaus, M.F. Zafeer, S. Ahmad, K. Akhtar, S.M. Dawar Husain, M.H. Ahmad, E. Anis, M. Mobarak Hossain, In vivo induction of antioxidant response and oxidative stress associated with genotoxicity and histopathological alteration in two commercial fish species due to heavy metals exposure in northern India (Kali) river, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 176-177 (2015) 17–30.
[30] J. Lourenço, R. Pereira, F. Gonçalves, S. Mendo, Metal bioaccumulation, genotoxicity and gene expression in the European wood mouse (Apodemus sylvaticus) inhabiting an abandoned uranium mining area, Sci. Total Environ. 443 (2013) 673–680.
[31] M. Rinaldi, A. Micali, H. Marini, E.B. Adamo, D. Puzzolo, A. Pisani, V. Trichilo, D. Altavilla, F. Squadrito, L. Minutoli, Cadmium, organ toxicity and therapeutic approaches, a review on brain, kidney and testis damage, Curr. Med. Chem. 24 (35) (2017) 3879–3893.
[32] V.V. Padma, R. Baskaran, S. Divya, L.B. Priya, S. Saranya, Modulatory effect of Tinospora cordifolia extract on Cd-induced oxidative stress in Wistar rats, Integr. Med. Res. 1 (2016) 48–55.
[33] C.H. Ramamurthy, A. Subastri, A. Suyavaran, K.C. Subbaiah, L. Valluru, C. Thirunavukkarasu, Solanum torvum Swartz. fruit attenuates cadmium-induced liver and kidney damage through modulation of oxidative stress and glycosylation, Environ. Sci. Pollut. Res. Int. 23 (8) (2016) 7919–7929.
[34] G. Gobe, D. Crane, Mitochondria, reactive oxygen species and cadmium toxicity in the kidney, Toxicol. Lett. 198 (1) (2010) 49–55.
[35] K. Sinha, P.B. Pal, P.C. Sil, Cadmium (Cd(2+)) exposure differentially elicits both cell proliferation and cell death related responses in SK-RC-45, Toxicol. In Vitro 28 (2) (2014) 307–318.
[36] R.K. Bao, S.F. Zheng, X.Y. Wang, Selenium protects against cadmium-induced kidney apoptosis in chickens by activating the PI3K/AKT/Bcl-2 signaling pathway, Environ. Sci. Pollut. Res. Int. 24 (25) (2017) 20342–20353.
[37] X.B. Song, G. Liu, Z.Y. Wang, L. Wang, Puerarin protects against cadmium-induced proximal tubular cell apoptosis by restoring mitochondrial function, Chem. Biol. Interact. 260 (2016) 219–231.
[38] C.A. Morais, V.V. de Rosso, D. Estadella, L.P. Pisani, Anthocyanins as inflammatory modulators and the role of the gut microbiota, J. Nutr. Biochem. 33 (2016) 1–7.
[39] S.S. Elshaer, H.M. Anwar, Relevance of megalin receptor injury with nuclear factor-kappa B upregulation in acute kidney injury induced in rats, J. Biochem. Mol. Toxicol. (2018), in press.
[40] Granger Joly, P. de Boissel, M. Fournier, J.C. Rodriguez-Lecompte, P. McKenna, F. Kibenge, A. Siah, Functional and molecular responses of the blue mussel Mytilus edulis' hemocytes exposed to cadmium - An in vitro model and transcriptomic approach, Fish Shellfish Immunol. 67 (2017) 575–585.