



Blackberry juice anthocyanidins limit cisplatin-induced renal pathophysiology in mice

Marcelo Gomes de Gomes^{a,b,*}, Lucian Del Fabbro^a, André Tiago Rossito Goes^a, Leandro Cattelan Souza^a, Franciele Donato^a, Silvana Peterini Boeira^a, Marina Prigol^a, Cristiano Ricardo Jesse^a

^a Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas, LaftamBio Pampa, Universidade Federal do Pampa, CEP 97650-000, Itaqui, RS, Brazil

^b Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Pampa, UNIPAMPA, Uruguaiana, RS, 97500-970, Brazil

ARTICLE INFO

Article history:

Received 29 January 2019

Received in revised form 12 April 2019

Accepted 18 April 2019

Keywords:

Cisplatin
Renal damage
Nutraceutical
Blackberry

ABSTRACT

Some studies have showed that intake of blackberry juice (BBJ) can prevent urinary tract infections. However, there is a lack of studies that evaluate the mechanisms by which BBJ has protective effect. Thus, the aim of current study was to evaluate the effects of BBJ supplementation on cisplatin-induced renal pathophysiology in mice. Mice were supplemented with BBJ (10 mL/kg) for seven days. One hour after the last supplementation with BBJ, mice received cisplatin (10 mg/kg, i.p.). Seventy-two hours after cisplatin administration, blood was collected and biochemical analysis were performed (urea and creatinine), kidney was dissected and utilized in histological and oxidative evaluations. Cisplatin caused severe injury in renal tissue, in markers of renal damage (urea and creatinine) generated increased of plasmatic levels. Besides that, the cisplatin induced decreased of enzymes activities in renal tissue (superoxide dismutase, glutathione S-transferase and catalase). In contrast, BBJ supplementation protected against histopathological alterations through decreased in urea and creatinine levels and modulation of catalase enzyme activity. Thus, BBJ supplementation protected the renal system of mice from deleterious effects. We suggest that high concentrations of Cyanidin 3-O-glucoside and Cyanidin 3-O-rutinoside are responsible for antioxidant role of BBJ supplementation in renal pathophysiology induced by cisplatin exposure. Also, these results reinforcing the importance of including BBJ in the human diet aimed at preventing renal diseases.

© 2019 Elsevier B.V. All rights reserved.

1. Introduction

Blackberry (*Rubus* spp.), belonging to the family Rosaceae, produces an aggregate fruit originating from many ovaries in a single flower and containing multiple small drupelets [1]. The Blackberry have different types of cultivars. Among several cultivars developed in Brazil through a breeding program for the adaptation to local climate and consumption, Tupy is currently the most sold [2]. Besides fresh consumption, the large market for products of blackberry is generated from its juice, which is used as a basis for developing a wide range of products such as jams, jelly, syrup, candy and wine [3].

Currently, blackberry have gained an important position in food industry due to presence of various substances with functional properties [4]. Blackberry are excellent material for the production of juice for human diet because they contain significant amount of phenolic compounds which have antioxidant properties and other health benefits [5]. The phenolic compounds were associated with different health benefits in cardiovascular diseases, cancer, diabetes, inflammatory processes and age-related neurodegenerative diseases [6,7].

In this context, blackberries are known to contain appreciable levels of phenolic compounds (± 241.7 mg/100 g of fruit) especially anthocyanins (± 90.5 mg/100 g of fruit), carotenoids (± 84.5 mg/100 g of fruit), and other bioactive compounds that can provide numerous health benefits [3,8]. Being the anthocyanins are probably the largest group of phenolic compounds in human diet [9]. The anthocyanins of blackberry have been used for various therapeutic purposes including inhibition of gastric and lung cancer in humans cells [10] and oxidative stress [11].

* Corresponding author at: Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas, LaftamBio Pampa, Universidade Federal do Pampa, CEP 97650-000, Itaqui, RS, Brazil.

E-mail address: marcelogomesdegomes@hotmail.com (M.G. de Gomes).

In view of the above, historically the blackberry juice (BBJ) has been consumed to prevent urinary tract infections (UTIs). The use of fruits juice preparations to prevent UTIs became popular in the 1920s when American scientists demonstrated that the urine became more acidic after eating large amounts of blackberry [12]. In this sense, the first event for development of a bacterium in body is that it binds in the cells/tissues of host. *Escherichia coli* strains that cause UTIs, have proteinaceous macromolecules (fimbriae) that facilitate the adhesion of bacterium to uroepithelial cells in urinary tract. *in vitro* and *ex vivo* studies have indicated that blackberry products prevented the adhesion of bacteria and virus to cell walls, suggesting that these products may be preventive against UTIs [13,14].

In view of foregoing, cisplatin (*cis*-dichlorodiammineplatinum II) is a synthetic anticancer drug extensively used in the treatment of several human malignancies. Anticancer activity of cisplatin is attributed to conversion a complex, which forms an interstrand cross-link with double-strand DNA to prevent DNA synthesis [15]. The most common adverse effect limiting the efficacy of this drug is nephrotoxicity which is developed primarily in S3 segment of proximal tubule. About 25–35% of patients experience a significant decline in renal function after a single dose of cisplatin [16]. The protective effect of various natural and synthetic antioxidant molecules, extracts, and foods have been investigated in different models of cisplatin-induced nephrotoxicity [17–22]. However, studies on the effect of blackberry consumption and its byproducts in models of renal damage induced by cisplatin are still scarce.

Thus, present study aimed to evaluate the effect of BBJ supplementation on cisplatin-induced renal pathophysiology in mice.

2. Materials and methods

2.1. Animals

Swiss albino mice (25–30 g, 70–90 days) were used. Animals were housed in groups of 5 in Plexiglas cages (41 cm × 34 cm × 16 cm). They were kept in a room with light-dark cycle of 12 h with the lights on between 7:00 and 19:00 h and temperature controlled (20–25 °C) and received water and food ad libitum. The animals were maintained and used in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources (process 028/2012) of the Federal University of Pampa, Brazil.

2.2. Chemicals

Cyaniding 3-*O*-glucoside, cyaniding 3-*O*-rutinoside, cisplatin, thiobarbituric acid (TBA), trichloroacetic acid (TCA), reduced glutathione (GSH), 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4 dinitrobenzene (CDNB), epinephrine bitartrate salt, glycine, tris, were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were obtained from standard commercial suppliers.

2.3. Obtaining of BBJ

Blackberry (*Rubus* spp.), Tupy cultivar, were collected from trees in the region (at University) and subsequently were fully subjected to a churning process in a blender for about six minutes. In order to get a whole juice, no part of material obtained was removed and any other ingredient was added. According to National Health Surveillance Agency [23], the juice is not fermented drinks, not concentrated and not diluted, for consumption, obtained from healthy and ripe fruit, or part of vegetable origin, appropriate technological processing.

2.4. Extraction and identification of anthocyanins

Ten grams of blackberry (*Rubus* spp.) fresh fruits, Tupy cultivar, were homogenized in 60 mL of 2% HCl methanol solution. Solution was filtered on a Büchner funnel, and the filtrate was used for HPLC analyses. Identifications were made by comparing PDA/UV, and retention data recorded for anthocyanin standards.

2.5. Quantitative analyses

Standard calibration curves were prepared over a concentration range for cyaniding 3-*O*-glucoside and cyaniding 3-*O*-rutinoside in a range 1–500 µg/mL with six different concentration levels (1, 5, 10, 50, 100, and 500 µg/mL). Triplicate injections were made for each level, and a weighed linear regression was generated for both external standards of calibration. The curve of calibration with external standards was obtained using concentration (µg/mL) with respect to area obtained from integration of PDA peaks. The relationship between variables was analyzed using linear simple correlation. For the linear regression of external standard, R^2 values was 0.9999 for cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside. For the quantification of compounds, GraphPad Software Prism 5.0 was used. The amount of compounds was expressed as milligrams per 10 g of dried fruits for flavonoids and milligrams per 10 g of fresh fruits for anthocyanins. PDA data were recorded over the 220–500 nm range with preferential channels 254 and 324 nm as detection wavelengths. In anthocyanin case, the eluent was a mixture of 1% acetonitrile solution of HCOOH (solvent A) and 1% aqueous solution of HCOOH (solvent B). Solvent gradient was as follows: 0–55 min, 5–50% (A). Elution was performed at a flow rate of 0.5 mL/min. Analyses were performed using a 3.0 × 150 mm, 3.5 µm, Symmetry C18 column (Waters). HPLC-PDA analyses were performed using a Waters 600E multisolvent delivery system, a Waters 717plus autosampler, and a Waters 996 PDA detector equipped with Millennium 32 Chromatography Manager software.

2.6. Experimental procedure

Mice were supplemented with BBJ (10 mL/kg) or saline solution 0.9% (w/v) (10 mL/kg, p.o.) for seven days. Sixty minutes after BBJ supplementation, mice were administered intraperitoneally (i.p.) with cisplatin (10 mg/kg), dissolved in saline solution 0.9% (w/v) [24,25]. The dose of BBJ supplementation was chosen according to the previous study [3]. The protocol of mice treatment is given below:

- Group 1 (Control): Saline + saline;
- Group 2 (BBJ): BBJ + saline;
- Group 3 (Cisplatin): Saline + cisplatin;
- Group 4 (BBJ + Cisplatin): BBJ + cisplatin.

72 h after cisplatin administration, according to methodology adapted from Wilhelm et al. [22], the mice were anesthetized, and blood was collected by cardiac puncture into tubes containing heparin (1 UI/µL). The kidney was removed, and one part was used for histopathological analysis and another part was homogenized in Tris–HCl 50 mM, pH 7.4, centrifuged at 2500 rpm for 10 min and the supernatant was used for determination of enzymatic or non-enzymatic indicators of oxidative stress.

2.7. Markers of renal damage

Urea and creatinine were measured in plasma using commercial Kits (Labtest, Diagnostica S.A., Minas Gerais, Brazil). Urea and creatinine levels were expressed as mg/dl.

2.8. Markers of oxidative stress

2.8.1. Ascorbic acid (AA) levels

Renal ascorbic acid determination was realized as described by Jacques-Silva et al. [26]. Protein of kidney was precipitated in 10 volumes of a cold 4% trichloroacetic acid solution. An aliquot of homogenized sample (300 mL), in a final volume of 1 mL of the solution, was incubated at 38 °C for 3 h, then 1 mL H₂SO₄ 65% (v/v) was added to medium. The reaction product was determined using color reagent containing 4.5 mg/mL dinitrophenyl hydrazine and CuSO₄ (0.075 mg/mL). AA levels were demonstrated as μmol ascorbic acid/g tissue.

2.8.2. Nonprotein thiols (NPSH) content

Renal NPSH levels were determined by method described of Eilman [27]. Supernatant was precipitated with 10% trichloroacetic acid and centrifuged at 3,000g for 10 min. Sulphydryl groups were determined using clear supernatant. An aliquot of supernatant was added in 1 M potassium phosphate buffer (pH 7.4) and 10 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). NPSH levels were measured spectrophotometrically at 412 nm and expressed as μmol NPSH/g tissue.

2.8.3. Lipid peroxidation (TBARS)

Renal lipid peroxidation was estimated by measuring of thiobarbituric acid reactive species (TBARS), according to method of Ohkawa et al. [28]. In this method, MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. TBARS content was estimated in a medium containing the supernatant fraction of kidneys 0.05 mL of 8.1% SDS, 0.2 mL of acetic acid buffer (2.5 M, pH 3.4), and 0.38 mL of 0.81% thiobarbituric acid (TBA). The mixture was finally made up to 1 mL with type I ultrapure water and heated at 95 °C for 90 min in a water bath using a glass ball as a condenser. After cooling to room temperature, absorbance was measured in the supernatant at 532 nm. Results were as expressed as nmol MDA/mg of protein.

2.8.4. Superoxide dismutase (SOD) activity

SOD activity in renal tissue was assayed spectrophotometrically as described [29]. This method is based on the capacity of SOD in inhibiting autoxidation of adrenaline to adrenochrome. Color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26 °C. SOD activity was expressed as Units/mg of protein.

2.8.5. Catalase (CAT) activity

Renal CAT activity was assayed spectrophotometrically by Aebi [30], which involves monitoring the disappearance of H₂O₂ in the presence of supernatant at 240 nm. Enzymatic reaction was initiated by adding an aliquot of S1 20 mL, and the substrate (H₂O₂) at a concentration of 0.3 mM in a medium containing 50 mM phosphate buffer, pH 7.0. CAT activity was expressed as Units/mg of protein.

2.8.6. Glutathione S-transferase (GST) activity

Renal GST activity was assayed spectrophotometrically at 340 nm by method of Habig [31]. The reaction mixture contained an aliquot of supernatant of liver, kidney or testes, 0.1 M potassium phosphate buffer (pH 7.4), 100 mM GSH and 100 mM CDNB, which

was used as substrate. Enzymatic activity was expressed as nmol CDNB/min/mg of protein.

2.9. Protein quantification

Renal protein concentration was measured by the method of Bradford [32], and bovine serum albumin was used as standard.

2.10. Histopathological analysis

The kidney was fixed in 10% formalin. For light microscopy examination, tissues were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin. Mice from all groups were examined by histopathology of parameters: Cellular vacuolization, loss of cellular architecture in the renal tubules and vascular congestion (n = 3 per group).

2.11. Statistical analysis

The data distribution was verified by applying Kolmogorov-Smirnov test. Results are presented as means ± standard error of the mean (S.E.M.). Statistical analysis was performed to compare treated groups to respective control groups using a two-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test when appropriate. Values of p < 0.05 were considered statistically significant.

3. Results

3.1. Quantitative analysis of anthocyanins profile

Anthocyanin composition of blackberry was determined by means of HPLC-PDA analyses. We identified two anthocyanins, cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside by comparison of their HPLC retention times, elution orders and photodiode array PDA/UV/VIS with authentic reference compounds. The total amount of anthocyanins is 190.2 mg/100 g of fresh berries of cyanidin 3-O-glucoside and 65.6 mg/100 g of fresh berries of cyanidin 3-O-rutinoside. BBJ supplementation group did not differ significantly from the control group in all analyzes (Fig. 1).

3.2. Markers of renal damage

Two-way ANOVA of plasma urea and creatinine levels revealed a significant BBJ X Cisplatin interaction ($F_{1,32} = 8.88$; $p < 0.01$) and ($F_{1,32} = 4.58$; $p < 0.05$). Bonferroni's multiple comparison test showed that acute administration of cisplatin induced a significant increase in plasma urea and creatinine levels ($F_{1,32} = 8.25$; $p < 0.01$) and ($F_{1,32} = 6.04$; $p < 0.05$) compared to control group. BBJ supplementation protected against the increased in urea and creatinine levels ($F_{1,32} = 10.70$; $p < 0.01$) and ($F_{1,32} = 5.04$; $p < 0.05$) respectively compared to cisplatin group (Fig. 2A and B).

3.3. Markers of oxidative stress

3.3.1. Ascorbic acid, NPSH and TBARS levels

Two-way ANOVA of AA, NPSH and TBARS levels in renal tissue showed a significant BBJ X Cisplatin interaction ($F_{1,32} = 4.67$; $p < 0.04$), ($F_{1,32} = 0.30$; $p > 0.05$) and ($F_{1,32} = 0.26$; $p < 0.04$) respectively. Bonferroni's multiple comparison test revealed no significant difference in AA, NPSH and TBARS levels in kidney tissue (Table 1).

3.3.2. SOD and GST activities

A Two-way ANOVA of SOD and GST activities in renal tissue showed a significant BBJ X Cisplatin interaction ($F_{1,32} = 6.07$;

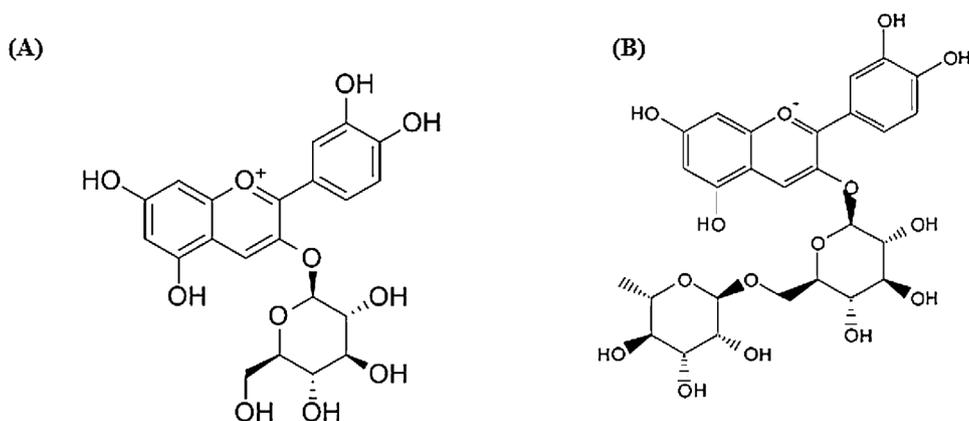


Fig. 1. Chemical structure of the main anthocyanins presents in black mulberries juice. (A). Chemical structure of cyanidin 3-O-glucoside (Present in concentration 190.2 mg/100 g of fresh berries). (B) Chemical structure of cyanidin 3-O-rutinoside (Present in concentration 5.6 mg/100 g of fresh berries).

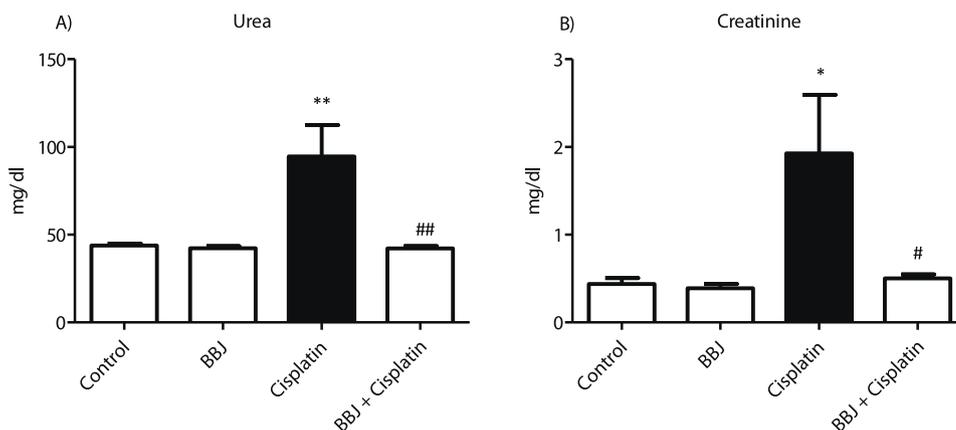


Fig. 2. (A). Effect of BBJ supplementation on plasmatic levels of urea in pathophysiology renal induced by cisplatin. (B). Effect of BBJ supplementation on plasmatic levels of creatinine in pathophysiology renal induced by cisplatin. Data are mean \pm S.E.M. for $n=8$ animals in each group. *Indicates a significant difference ($p < 0.05$) compared with control group. **Indicates a significant difference ($p < 0.01$) compared with control group. #Indicates a significant difference ($p < 0.05$) compared with cisplatin group. ##Indicates a significant difference ($p < 0.01$) compared with cisplatin group.

Table 1
Effect of BBJ supplementation on ascorbic acid, NPSH and TBARS levels in renal tissue of mice exposed to cisplatin.

Groups	Ascorbic acid (μg ascorbic acid/g tissue)	NPSH (μmol GSH/g tissue)	TBARS (nmol MDA equivalents/g tissue)
Control	4,02 \pm 0,79	25,80 \pm 3,95	25,96 \pm 5,41
BBJ	4,64 \pm 0,53	20,29 \pm 6,37	22,55 \pm 6,87
Cisplatin	4,75 \pm 0,84	22,54 \pm 3,40	24,18 \pm 6,08
BBJ + Cisplatin	4,62 \pm 1,12	20,26 \pm 4,78	22,58 \pm 5,65

The animals were supplemented with BBJ (10 ml/kg) for seven days. Sixty minutes after supplementation with BBJ, mice were administered with cisplatin (10 mg/kg, i.p.). Data are expressed as means \pm S.E.M of 8 animals per group. * $P < 0,05$ when compared with control group. ** $P < 0,01$ when compared with control group. # $P < 0,05$ when compared with cisplatin group.

Table 2
Effect of BBJ supplementation on SOD, GST and CAT activities in renal tissue of mice exposed to cisplatin.

Groups	SOD units/mg protein	GST nmol CDNB conjugated/min/mg protein	CAT units/mg protein
Control	2,21 \pm 0,76	0,44 \pm 0,10	2,40 \pm 0,27
BBJ	1,45 \pm 0,49	0,30 \pm 0,10	2,49 \pm 0,92
Cisplatin	1,43 \pm 0,34*	0,24 \pm 0,13**	1,49 \pm 0,31*
BBJ + Cisplatin	1,56 \pm 0,54*	0,22 \pm 0,11**	2,27 \pm 0,50#

The animals were supplemented with BBJ (10 ml/kg) for seven days. Sixty minutes after supplementation with BBJ, mice were administered with cisplatin (10 mg/kg, i.p.). Data are expressed as means \pm S.E.M of 8 animals per group.

* $P < 0,05$ when compared with control group.

** $P < 0,01$ when compared with control group.

$P < 0,05$ when compared with cisplatin group.

$p < 0,02$) and ($F_{1,32} = 2.39$; $p > 0,05$). Bonferroni's multiple comparison test showed that acute administration of cisplatin induced a significant decreased in SOD and GST activities in kidney

($F_{1,32} = 3.41$; $p < 0.05$) and ($F_{1,32} = 14.73$; $p < 0.01$) compared to control group. The supplementation with BBJ did not modify the reduction of activities SOD and GST in renal tissue ($F_{1,32} = 2.97$;

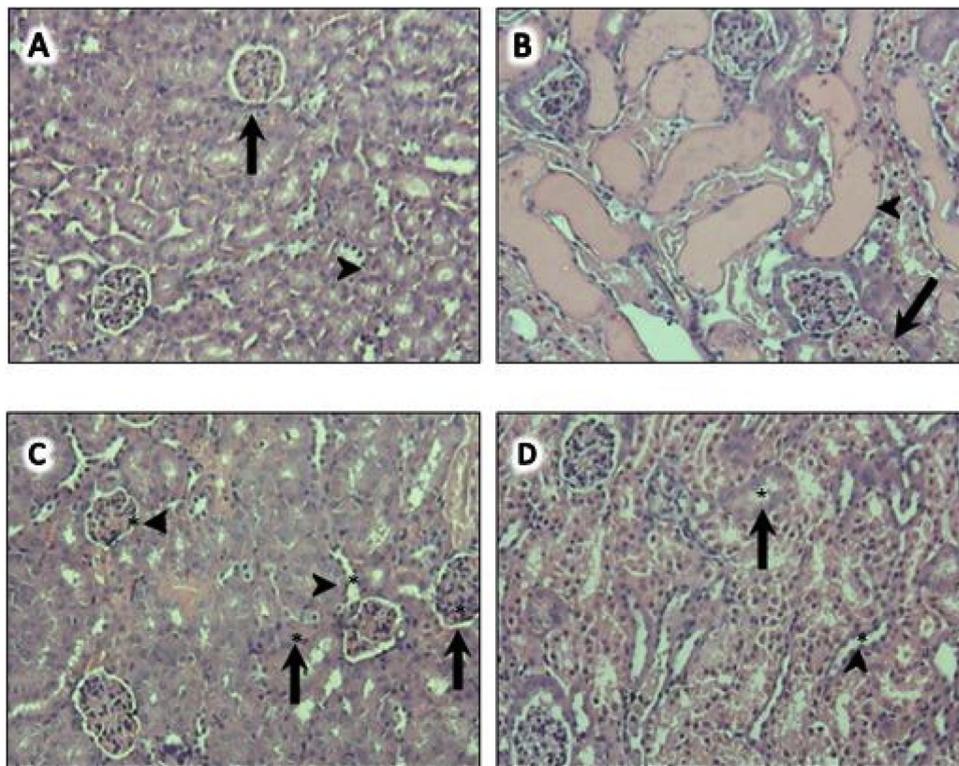


Fig. 3. (A). Photomicrography of segment of the renal lobe of an animal control. (B). Photomicrography of an animal treated with cisplatin showed necrosis. (C). Photomicrography of an animal supplemented with BBJ at the dose of 10 mL/kg. (D). Photomicrography of an animal treated with BBJ + cisplatin. Note the renal tissue destructuring and cytoplasmatic vacuolization (*). (arrow), Proximal Tubule; (arrow-head), Distal Tubule. (H.E). 100 × .

$p > 0.05$) and ($F_{1,32} = 5.10$; $p > 0.05$) compared to cisplatin group (Table 2).

3.3.3. CAT activity

Two-way ANOVA of CAT activity in renal tissue showed a significant BBJ X Cisplatin interaction ($F_{1,32} = 2.85$; $p > 0.05$). The Bonferroni's multiple comparison test showed that acute administration of cisplatin induced a significant decreased in CAT activity in renal tissue ($F_{1,32} = 7.70$; $p < 0.05$) compared to control group. BBJ supplementation protected against the reduction of activity CAT in renal tissue ($F_{1,32} = 4.50$; $p < 0.05$) compared to cisplatin group (Table 2).

3.4. Histopathological analysis

Histological evaluation revealed a normal aspect of kidney structures in control and BBJ supplementation groups (Fig. 3A and B). Mice exposed to cisplatin presented intense loss of cellular architecture, with extensive injuries, cellular vacuolization, severe proximal and distal tubular damage and marked vascular congestion between tubules, and cellular vacuolization (Fig. 3C). Evaluation of kidneys from mice exposed to BBJ supplementation and cisplatin showed tubules with histological characteristics more preserved than those from the cisplatin group. Renal tubules displayed moderate damage. BBJ markedly ameliorated the degree of kidney damage (Fig. 3D).

4. Discussion

Functional food is one that acts in body not only in nutritional functions, but also contributing in quality of life and disease prevention [33,34]. In this context, blackberry and its byproducts have gained an important position in the food industry due to presence

of various substances with functional properties, mainly anthocyanins [34].

In our study, we found high levels of anthocyanins in BBJ such as cyanidin 3-O-glucoside (190,2 mg/100 g) and cyanidin 3-O-glucoside (65,6 mg/100 g). These results are consistent with the data quoted by Hager [35], Wang et al. [36] and Ferreira et al. [4], who described cyanidin 3-glucoside as the predominant anthocyanins in blackberries, observing concentrations ranging from 67.4 to 248.0 mg/100 g.

In this study, the renal damage induced by acute administration of cisplatin was evidenced by an increase in plasmatic levels of urea and creatinine, besides histological changes and decreased of SOD, GST and CAT activities in renal tissue. Protective effect of BBJ supplementation against renal pathophysiology induced by cisplatin was demonstrated via protection against increased in plasmatic urea and creatinine levels. Still, preventing histopathological changes and protecting against decreased of CAT activity in renal tissue.

In this sense, renal pathophysiology induced by acute cisplatin was evidenced by significant increase in plasmatic levels of urea and creatinine, because in physiological conditions these substances are continuously excreted in urine. Therefore, increased in plasmatic urea and creatinine levels is indicative of flaws in the physiological processes of kidneys. The supplementation with BBJ significantly protected against increases in plasmatic levels urea and creatinine showing nephroprotective effect of BBJ. In a similar study, Wilhelm et al. [22] also found a significant increased in markers of renal damage. Other studies have reported protective effect of various substances, extracts, and foods against renal damage induced by cisplatin in rodents. Sultana et al. [37] showed the protective effect of administration of chrysin, a natural flavonoid, for 14 days in rats. Sharma and Goyal [38], showed nephroprotective effect by methanolic extract of *Heliotropium eichwaldii* in mice.

Naqshbandi et al. [39] reported protective effect by administration of fish oil for 10 days in rats.

Histopathological analysis corroborates demonstrating the pathophysiology induced by cisplatin. The group treated with cisplatin showed a high number of lesions in kidney tissue, presenting intense loss of cellular architecture, severe proximal and distal tubular damage, marked vascular congestion between tubules, and cellular vacuolization. BBJ supplementation protected against the incidence and severity of these lesions reiterating the nephroprotective effect. Other authors such as Yildirim et al. [17], Ozer et al. [21] and Wilhelm et al. [22] have reported the occurrence of lesions in renal tissue after acute exposition of cisplatin in rodents.

No changes were found for TBARS, AA and NPSH levels in renal tissue. TBARS levels are a biomarker of lipid peroxidation resulting from oxidative stress. Similarly, Iseri et al. [40] administered cisplatin in rats and also did not find significant differences in TBARS levels. On the other hand, Yousef et al. [41] performed a similar protocol for induction of oxidative stress by administration of cisplatin in rats and observed a significant increase in TBARS levels in kidney tissue. The maintenance of AA levels in renal tissue showed that altering levels of AA was not one of mechanisms involved in renal pathophysiology induced by cisplatin in our study. Studies in mice, rats and humans have showed that AA is essential to keep adequate levels of glutathione, which is the stable form of cysteine [42–44]. Thus, due the AA relation with glutathione, we suggest that maintenance of AA levels in renal tissue observed in our study may be related to unchanged TBARS and NPSH levels.

Also, the maintenance of NPSH levels found in our study appear is not mediating cisplatin-induced renal damage. Naghizadeh et al. [45] and Wilhelm et al. [22] evaluated NPSH levels in renal tissue of rats and mice respectively, after the administration of cisplatin and observed a significant reduction in NPSH levels. These results are contrary to those obtained in our study. Reduced glutathione (GSH) is an important antioxidant and represents approximately 90% of NPSH levels. GSH makes a pivotal role in detoxification of a variety of electrophilic compounds and peroxides via catalysis by glutathione S-transferases (GST) and glutathione peroxidases (GPx). Thus, given important role of thiol groups in antioxidant defenses, the balance of adequate levels of NPSH may be involved with maintenance of normal levels of TBARS observed in this study. In addition, factors such as species, strain, dose, and time among treatment with cisplatin and euthanasia may be related to any difference of the results of TBARS, AA and NPSH levels between aforementioned studies and our study.

In this context, treatment with cisplatin significantly decreased SOD activity in renal tissue compared with control group. SOD is an enzyme essential for all aerobic cells for catalyzing the dismutation of superoxide radical forming H_2O_2 and O_2 [46,47]. Thus, considering the importance of SOD for physiological processes in kidney, the decreased of SOD activity observed in our study is probably one of the mechanisms involved in renal pathophysiology cisplatin-induced in mice. Supplementation with BBJ did not protect against the decreased of SOD activity showing that this enzyme is not related to effect of BBJ.

Consequently, the GST activity was significantly decreased in cisplatin group. Sahu et al. [48] and Wilhelm et al. [22] also evaluated the activity of GST in renal tissue from rats after the administration of cisplatin and found a results similar to ours. It is known that GST is an important group of proteins involved in detoxification of electrophilic compounds, found intracellularly or in form of xenobiotics [49]. Thus, the reduction in activity of GST caused by cisplatin administration shows that alteration in enzyme activity is one of the mechanisms by which cisplatin generated nephrotoxicity. BBJ supplementation did not modulate GST activity altered by cisplatin.

For CAT activity, when cisplatin was administered caused significantly decreased in activity. The supplementation with BBJ significantly protected against the decreased in CAT activity. Decreased of CAT activity in renal tissue observed in our study is one of the mechanisms involved in renal pathophysiology cisplatin-induced, corroborating the findings by Ozer et al. [21] and Wilhelm et al. [22]. In this context, the protection of decreased in CAT activity is pivotal role for protection by BBJ supplementation against the renal pathophysiology caused by cisplatin. This nephroprotective effect of BBJ supplementation shows that prevention of reduction of CAT activity, is one of the mechanisms by which BBJ modulates the deleterious effects caused by cisplatin in renal tissue. Confirming the importance of regulation of CAT activity as a protective mechanism in models of renal damage, Ibrahim et al. [50] showed the role of CAT in a model of renal injury induced by glycerol in rats. Furthermore, Ozer et al. [22] and Wilhelm et al. [23] demonstrated the role of CAT in a model of renal damage induced by cisplatin in rats and mice, respectively.

In view of the foregoing, the bioactive effect of blackberry is attributed to its high concentration of anthocyanins [2]. According to Rice-Evans et al. [51], anthocyanins acts as an antioxidant donating hydrogen to free radicals. Kaume et al. [3] affirmed that cyanidin 3-glucoside and cyanidin 3-rutinoside are the main anthocyanin compounds present in blackberry in quantitative terms. In a similar study in aged rats, Shukitt-Hale et al. attributed that the neuroprotection caused by blackberry was due to its compounds cyanidin 3-glucoside and cyanidin 3-rutinoside [52]. Several authors such as Manach et al. [53], Ferreira et al. [4] and Virgili and Marino [54] have reported the antioxidant properties of anthocyanins from blackberries. Therefore, the anthocyanins present on BBJ supplementation may be modulating the pharmacological effects presented in our study.

5. Conclusion

In this contribution, we have showed that BBJ supplementation protected against renal pathophysiology induced by cisplatin in mice. Still, the anthocyanins present in BBJ supplementation seem to be mediating the pharmacological effects. Therefore, the results presented in this study added data on functional properties of BBJ, reinforcing the importance of including BBJ in human diet aimed at preventing renal diseases.

Conflict of Interest

The authors declare that there are no conflicts of interest in the present work.

Acknowledgements

The financial support by CAPES, CNPq and FAPERGS is gratefully acknowledged. This study was financed in part by the Coordination for the Improvement of Higher Education Personnel- Brazil (CAPES) – Finance Code 001. Also to all the members of LaftamBio Pampa and especially the Master.

References

- [1] C. Zhang, Z. Xiong, H. Yang, W. Wu, Changes in pericarp morphology, physiology and cell wall composition account for flesh firmness during the ripening of blackberry (*Rubus* spp.) fruit, *Sci. Hortic.* 250 (2019) 59–68.
- [2] A. Zielinski, A. Ferreira, C. Goltz, M. Yamato, A. Casagrande, S. Ávila, R.Y. Hirooka, G. Wosiacki, A. Nogueira, I.M. Demiate, Blackberry (*Rubus* spp.): influence of ripening and processing on levels of phenolic compounds and antioxidant activity of the 'Brazos' and 'Tupy' varieties grown in Brazil, *Rural Sci.* 45 (2015) 744–749.

- [3] L. Kaume, L.R. Howard, L. Devareddy, The blackberry fruit: a review on its composition and chemistry, metabolism and bioavailability, and health benefits, *J. Agric. Food Chem.* 60 (2012) 5716–5727.
- [4] D.S. Ferreira, V.V. de Rosso, A.Z. Mercadante, Bioactive compounds of blackberry fruits (*Rubus spp.*) grown in Brazil, *Braz. J. Fruticult.* 32 (2010) 664–674.
- [5] T.S. Kahlon, G.E. Smith, In vitro binding of bile acids by blueberries (*Vaccinium spp.*), plums (*Prunus spp.*), prunes (*Prunus spp.*), strawberries (*Fragaria ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*), *Food Chem.* 100 (2007) 1182–1187.
- [6] J. He, M. Giusti, Anthocyanins: natural colorants with health-promoting properties, *Annu. Rev. Food Sci. Technol.* 1 (2010) 163–187.
- [7] G. Azofeifa, S. Quesada, L. Navarro, O. Hidalgo, K. Portet, A.M. Pérez, F. Vaillant, P. Pouchet, A. Michel, Hypoglycaemic, hypolipidaemic and antioxidant effects of blackberry beverage consumption in streptozotocin-induced diabetic rats, *J. Funct. Foods* 26 (2016) 330–337.
- [8] M.J. Cho, L.R. Howard, R.L. Prior, J.R. Clark, Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry, *J. Sci. Food Agric.* 85 (2005) 2149–2158.
- [9] A. Cassidy, M. Franz, E.B. Rimm, Dietary flavonoid intake and incidence of erectile dysfunction, *Am. J. Clin. Nutr.* 103 (2016) 534–541.
- [10] O. Paredes-López, M. Cervantes-Ceja, M. Vigna-Pérez, T. Hernández-Pérez, Berries: improving human health and healthy aging, and promoting quality life-review, *Plant Foods Hum. Nutr.* 65 (2010) 299–308.
- [11] H.A. Hassan, H.M. Serage, W. Gad, Black berry juice attenuates neurological disorders and oxidative stress associated with concurrent exposure of aluminum and fluoride in male rats, *Egypt. J. Basic Appl. Sci.* 2 (2015) 281–288.
- [12] R.G. Jepson, J.C. Craig, A systematic review of the evidence for cranberries and blueberries in UTI prevention, *Mol. Nutr. Food Res.* 51 (2007) 738–745.
- [13] R.J. Danaher, C. Wang, J. Dai, R.J. Mumper, C.S. Miller, L. Kentucky, C. Hill, Antiviral effects of blackberry extract against herpes simplex virus type 1, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontology* 112 (2011) 31–35.
- [14] S. Yilmaz, Effects of dietary blackberry syrup supplement on growth performance, antioxidant, and immunological responses, and resistance of Nile tilapia, *Oreochromis niloticus* to *Plesiomonas shigelloides*, *Fish Shellfish Immunol.* 84 (2019) 1125–1133.
- [15] S.M. Baek, C.H. Kwon, J.H. Kim, J.S. Woo, J.S. Jung, Y.K. Kim, Differential roles of hydrogen peroxide and hydroxyl radical in cisplatin-induced cell death in renal proximal tubular epithelial cells, *J. Lab. Clin. Med.* 142 (2003) 178–186.
- [16] D.R. Luke, K. Vadieli, G. Lopez-Berestein, Role of vascular congestion in cisplatin-induced acute renal failure in the rat, *Nephrol. Dial. Transplant.* 7 (1992) 1–7.
- [17] Z. Yildirim, S. Sogut, E. Odaci, M. Iraz, H. Ozyurt, M. Kotukm, O. Akyol, Oral erdoesteine administration attenuates cisplatin-induced renal tubular damage in rats, *Pharmacol. Res.* 47 (2003) 149–156.
- [18] M. Naziroğlu, A. Karaoğlu, A.O. Aksoy, Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats, *Toxicology* 195 (2004) 221–230.
- [19] D.M. Maliakel, T.V. Kagiya, C.K.K. Nair, Prevention of cisplatin-induced nephrotoxicity by glucosides of ascorbic acid and α -tocopherol, *Exp. Toxicol. Pathol.* 60 (2008) 521–527.
- [20] M.A.C. Rodrigues, J.L. Rodrigues, N.M. Martins, F. Barbosa, C. Curti, N.A.G. Santos, A.C. Santos, Carvedilol protects against the renal mitochondrial toxicity induced by cisplatin in rats, *Mitochondrion* 10 (2010) 46–53.
- [21] M.K. Ozer, H. Ascı, M. Oncu, M. Calapoglu, M. Savran, S. Yesilot, I.A. Candan, E. Cicek, Effects of misoprostol on cisplatin-induced renal damage in rats, *Food Chem. Toxicol.* 49 (2011) 1556–1559.
- [22] E.A. Wilhelm, C.F. Bortolotto, C.W. Nogueira, p-Methoxyl-diphenyl diselenide protects against cisplatin-induced renal toxicity in mice, *Food Chem. Toxicol.* 50 (2012) 1187–1193.
- [23] National Health Surveillance Agency, Decree number. 4, 1997, pp. 2314.
- [24] T.A. Ajith, S. Usha, V. Nivitha, Ascorbic acid and alpha-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice. A comparative study, *Clin. Chim. Acta* 375 (2007) 82–86.
- [25] A.A. Fouad, M.A. Morsy, W. Gomaa, Protective effect of carnosine against cisplatin-induced nephrotoxicity in mice, *Environ. Toxicol. Pharmacol.* 25 (2008) 292–297.
- [26] M.C. Jacques-Silva, C.W. Nogueira, L.C. Broch, E.M.M. Flores, J.B.T. Rocha, Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice, *Pharm. Toxicol.* 88 (2001) 119–125.
- [27] G.L. Ellman, Tissue sulfhydryl groups, *Arch. Biochem. Biophys.* 82 (1959) 70–77.
- [28] H. Ohkawa, N. Ohish, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem.* 95 (1979) 351–358.
- [29] H.P. Misra, I. Fridovich, The generation of superoxide radical during the autoxidation of hemoglobin, *J. Biol. Chem.* 247 (1972) 6960–6962.
- [30] U. Aebi, W. Chiu, R. Milligan, Role of catalase on antioxidative defenses, *J. Struct. Biol.* 1 (1984) 117–118.
- [31] W.H. Habig, M.J. Pabst, G. Fleishner, Z. Gatmaitan, I.M. Arias, W.B. Jacoby, The identity of glutathione S-transferase B with ligandin, a major binding protein of liver, *Proc. Natl. Acad. Sci. U. S. A.* 71 (1974) 3879–3882.
- [32] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- [33] P.G.B. de Carvalho, C.M.M. Machado, C.L. Moretti, M.E.N. Fonseca, Vegetables as functional foods, *Braz. Horticul.* 24 (2006) 397–404.
- [34] F.P. Moraes, L.M. Colla, Functional foods and nutraceuticals: definitions, legislation and health benefits, *Electron. J. Pharm.* 3 (2006) 109–122.
- [35] T. Hager, L.R. Howard, R.L. Prior, Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products, *J. Agric. Food Chem.* 56 (2008) 689–695.
- [36] S.Y. Wang, L. Bowman, M. Ding, Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus sp.*) and promotes antiproliferation of human cancer cells, *Food Chem.* 107 (2008) 1261–1269.
- [37] S. Sultana, K. Verma, R. Khan, Nephroprotective efficacy of chrysin against cisplatin-induced toxicity via attenuation of oxidative stress, *J. Pharm. Pharmacol.* 64 (2012) 872–881.
- [38] S.K. Sharma, N. Goyal, Protective effect of Heliotropium eichwaldi against cisplatin-induced nephrotoxicity in mice, *J. Chin. Integr. Med.* 10 (2012) 555–560.
- [39] A. Naqshbandi, M.W. Khan, S. Rizwan, S.U. Rehman, F. Khan, Studies on the protective effect of dietary fish oil on cisplatin induced nephrotoxicity in rats, *Food Chem. Toxicol.* 50 (2012) 265–273.
- [40] S. İşeri, F. Ercan, N. Gedik, M. Yüksel, I. Alican, Simvastatin attenuates cisplatin-induced kidney and liver damage in rats, *Toxicology* 230 (2007) 256–264.
- [41] M.I. Yousef, A.A. Saad, L.K. El-Shennawy, Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats, *Food Chem. Toxicol.* 47 (2009) 1176–1183.
- [42] C.S. Johnston, C.G. Meyer, J.C. Srilakshmi, Vitamin C elevates red blood cell glutathione in healthy adults, *Am. J. Clin. Nutr.* 58 (1993) 103–105.
- [43] A. Meister, Glutathione-ascorbic acid antioxidant system in animals, *J. Biol. Chem.* 269 (1994) 9397–9400.
- [44] G. Paolisso, V. Balbi, C. Volpe, G. Varricchio, A. Gambardella, F. Saccomanno, S. Ammendola, M. Varricchio, F. D'Onofrio, Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics, *J. Am. Coll. Nutr.* 14 (1995) 387–392.
- [45] B. Naghizadeh, S. Mohammad, T. Mansouri, N.V. Mashhadian, Crocin attenuates cisplatin-induced renal oxidative stress in rats, *Food Chem. Toxicol.* 48 (2010) 2650–2655.
- [46] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs, *Physiol. Rev.* 59 (1979) 527–605.
- [47] P.A. Southorn, G. Powis, Free radicals in medicine I. Nature and biologic reactions, *Mayo Clin. Proc.* 63 (1988) 381–389.
- [48] B.D. Sahu, K.K. Rentam, U.K. Putcha, M. Kuncha, G.M.N. Vegi, R. Sista, Carnosic acid attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity, *Food Chem. Toxicol.* 49 (2011) 3090–3097.
- [49] M.C.L. Torres, N. Soares, J.A.M. Pereira, Extraction of glutathione s-transferase from bovine liver, *Agrotechnol. Sci.* 30 (2006) 302–307.
- [50] M. Ibrahim, G. Luchese, S. Pinton, S.S. Roman, W. Hassan, C.W. Nogueira, J.B.T. Rocha, Involvement of catalase in the protective effect of binaphthyl diselenide against renal damage induced by glycerol, *Exp. Toxicol. Pathol.* 63 (2010) 331–335.
- [51] C.A. Rive-Evans, N.J. Miller, G. Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids, *Free Radic. Biol. Med.* 20 (1996) 933–956.
- [52] B. Shukitt-Hale, V. Cheng, J.A. Joseph, Effects of blackberries on motor and cognitive function in aged rats, *Nutr. Neurosci.* 12 (2009) 135–140.
- [53] C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Remesy, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, *Am. J. Clin. Nutr.* 81 (2005) 230–242.
- [54] F. Virgili, M. Marino, Regulation of cellular signals from nutritional molecules: a specific role for phytochemicals, beyond antioxidant activity, *Free Radic. Biol. Med.* 45 (2008) 1205–1216.