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Protective effect of bixin on cisplatin-induced genotoxicity in PC12 cells

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ABSTRACT

Bixin is the main carotenoid found in annatto seeds (*Bixa orellana* L.) and is responsible for their reddish-orange color. The antioxidant properties of this compound are associated with its ability to scavenge free radicals, which may reduce damage and protect tissues against toxicity caused by anticancer drugs such as cisplatin. In this study, the genotoxicity and antigenotoxicity of bixin on cisplatin-induced toxicity in PC12 cells was assessed. Cytotoxicity was evaluated using the MTT assay, mutagenicity, genotoxicity, and protective effect of bixin were evaluated using the micronucleus test and comet assay. PC12 cells were treated with bixin (0.05, 0.08, and 0.10 μg/mL), cisplatin (0.1 μg/mL) or a combination of both bixin and cisplatin. Bixin was neither cytotoxic nor genotoxic compared to the controls. In the combined treatment bixin significantly reduced the percentage of DNA in tail and the frequency of micronuclei induced by cisplatin. This result suggests that bixin can function as a protective agent, reducing cisplatin-induced DNA damage in PC12 cells, and it is possible that this protection could also extend to neuronal cells. Further studies are being conducted to better understand the mechanisms involved in the activity of this protective agent prior to using it therapeutically.

1. Introduction

Carotenoids are a family of fat-soluble pigments found in fruits and vegetables. They are the most potent suppressors of biological singlet O_2 (Di Mascio et al., 1989). Carotenoids interact with singlet O_2 either via physical suppression, in which the energy of the excited singlet O_2 molecule is transferred to the carotenoid and then dissipated as heat, or by chemical suppression mechanisms, in which the carotenoid is destroyed through the addition of O_2 to its double-bond system (Liebler, 1993). The antioxidant properties of carotenoids have been suggested to be responsible for the main beneficial effects associated with these compounds (Rao and Rao, 2007). These effects include a decreased risk of developing sores and cataracts as well as several chronic degenerative diseases such as cancer, cardiovascular disease and macular degeneration (Lampe, 1999; Stanner et al., 2004).

Bixin is the main carotenoid found in the seeds of annatto trees (*Bixa orellana* L.), which are native to tropical America and are cultivated in the tropical regions of Central and South America, Africa and Asia (Mercadante et al., 1997). This carotenoid is responsible for the orange-red coloration of annatto seeds and their extracts (Chisté et al., 2011). Annatto extract is widely used in the food industry as a natural colorant in many formulations, including ice cream, cheese, sausage, yogurt and margarine. It is also used by the pharmaceutical and cosmetic industries (Rios et al., 2007).

Among the carotenoids, bixin is an efficient suppressor of singlet oxygen in fluid solutions, with a molecular suppression rate similar to that observed with β-carotene (Manitto et al., 1987; Rios et al., 2007). β-Carotene may be involved in protecting cells and tissues against the deleterious effects of free radicals, which cause oxidative damage to DNA (Antunes et al., 2005; Collins, 2001).

Cisplatin [*cis*-diamminedichloroplatinum(II), cDDP] is one of the most potent chemotherapeutic agents available and is highly effective at treating several types of cancer, including ovarian,

Abbreviations: BN, binucleated cells; CBPI, cytokinesis block proliferating index; cDDP, cisplatin; CNS, central nervous system; DMSO, dimethylsulfoxide; FACS, fluorescence activated cell sorting; MN, micronucleus; PC12, pheocromocytoma from the adrenal glands of rats; ROS, reactive oxygen species; SCGE, single-cell gel electrophoresis.

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bladder, lung, cervical, and testicular (Rabik and Dolan, 2007; Wang and Lippard, 2005). However, treatment with cisplatin and its analogs is often hindered by side effects, including nephrotoxicity, peripheral neurotoxicity, and ototoxicity (Knight et al., 2005). While diuretics and the pre-hydration of patients undergoing treatment have been shown to successfully control nephrotoxicity, peripheral neurotoxicity remains as the principal dose-limiting toxic effect (Lichtman et al., 2007; Polycarpe et al., 2004). The neurological effects of cisplatin may be severe and affect the patient's quality of life, even long after treatment ends (Pace et al., 2003).

Cisplatin is capable of generating reactive oxygen species (ROS) such as superoxide and hydroxyl radicals (Yoshida et al., 2003). It has also been shown that chemotherapy with cisplatin causes a decrease the plasma concentrations of various antioxidants (Weijl et al., 1998). This decrease can lead to a failure of the antioxidant defense mechanisms against organ damage, which are mediated by free radicals, and the subsequent mutagenic capability of cisplatin. The genotoxicity of antitumor drugs to normal tissues can lead to the induction of secondary malignancies. Several studies have shown that the administration of antioxidants can reduce the side effects associated with cisplatin (Antunes et al., 2000; Leonetti et al., 2003; Pace et al., 2003; Weijl et al., 1997; Wozniak et al., 2004; Yoshida et al., 2003), including neurotoxicity.

One of neuronal cell lines most frequently used to study neuronal function is the cell line PC12 (pheocromocytoma from the adrenal glands of rats). These cells have many advantages over primary cultures of neuronal cells, including the homogeneity of the cell population (Piga et al., 2007). In our study, this neuronal cell line was used to evaluate the possible cytotoxicity and genotoxicity of bixin as well as its protective effects against cisplatin-induced genotoxic damage.

2. Materials and methods

2.1. Preparation of samples and reagents

Dry annatto seeds were collected in the city of Ribeirão Preto, São Paulo, Brazil. Bixin was extracted from annatto seeds washed with ethyl acetate, hexane and methanol. The solvents were completely evaporated in a roto-evaporator (Büchi Labortechnik), and crystallization was performed with dichloromethane/ethanol (1:4). Bixin crystals were formed with 95% purity and were stored under refrigeration and protected from light until use (Rios and Mercadante, 2004).

Bixin was dissolved in dimethylsulfoxide (DMSO, Sigma Chemical Co., USA) at a final concentration of 0.4%. All solutions containing bixin were prepared immediately prior to use. Cisplatin (cDDP; CAS No. 15663-27-1) was acquired from Quiral Química do Brasil S.A. (Platinil®). All other reagents were of high purity.

2.2. Cell line

A rat pheochromocytoma cell line (PC12 cells) from the American Type Culture Collection (Rockville, MD, USA) was used in all experiments. The cells were cultured in RPMI 1640 medium (Sigma–Aldrich Co.) at 37 °C in a humidified incubator with an atmosphere of 95% air and 5% CO_2 (Thermo Electron Co.). The medium contained 10% heat-inactivated horse serum (Invitrogen, Brazil), 5% heat-inactivated fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin antibiotic mixture (Invitrogen). In all experiments, the cells were incubated 24 h before beginning bixin and/or cisplatin treatments.

2.3. MTT assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (a tetrazolium salt reduction assay) provides sensitive measurements of the normal metabolic status of cells, particularly that of the mitochondrion, where measurements reflect early cellular redox changes (Mosmann, 1983). PC12 cells (1×10^5 cells/well in 96-well plates) were incubated at 37 °C for 24 h with different concentrations of bixin (0.05–20 μg/mL), cDDP (0.1 μg/mL) or a combination of both (bixin + cDDP). A negative control containing only cells and a solvent control (cells treated with 0.4% DMSO) were also evaluated. After treatment, the plates were incubated in the MTT solution (final concentration of 0.5 mg/mL) for 3 h. The dark-blue formazan crystals that formed in intact cells were dissolved with DMSO, and the absorbance at 570 nm was measured with a microenzyme-linked immunosorbent assay (ELISA) reader (Sigma). The results were expressed as the percentage of MTT reduction relative to the absorbances measured from negative control cells. All assays were performed in triplicate.

2.4. Micronucleus test

The *in vitro* micronucleus (MN) test was conducted as described by Fenech (2000) with slight modification. Cells (1×10^6 in a 25 cm^2 flask) were incubated 24 h before treatment with cDDP (0.1 μg/mL), bixin (0.05, 0.08, or 0.10 μg/mL) or a combination of both (bixin + cDDP); in the combined treatments, cisplatin was added to the culture medium 1 h after bixin was added (25 h after the initial incubation of the cells), and all treatments were set until harvest. The concentrations of bixin used were established based on the results of the MTT assay (concentrations of bixin with cell viability above 80%). A negative control (untreated culture) and solvent control (culture of 0.4% DMSO-treated cells) were also evaluated.

Cytochalasin B (6 μg/mL) was added to arrest cytokinesis after 44 h of growth, and the cells were harvested and placed in a cold hypotonic solution (1% sodium citrate) and fixed three times in cold methanol:acetic acid (3:1) 72 h after initiating the culture. The initial addition of fixative contained 1% formaldehyde, which enhances cytoplasm preservation. Slides were prepared and stained in a solution of 4% Giemsa dye (Sigma–Aldrich Co.) in phosphate buffer (pH 6.8) for 10 min. The slides were coded and scored by light microscopy at 400× or 1000× magnification, as required. In three independent experiments, 2000 binucleated cells (BN) with well-preserved cytoplasms were scored.

MN cells were identified according to the criteria previously published by Fenech et al. (2003). The frequency of binucleated cells containing one or more MN was also determined. As a measure of cell proliferation, the cytokinesis block proliferating index (CBPI) was calculated according to the following formula: CBPI = [MI + 2-MII + 3(MIII + MIV)]/*N*, as proposed by Surrallés et al. (1995), where MI–MIV represents the number of cells with 1–4 nuclei in a total of 500 cells. The capacity of the bixin reduces the damage caused by cDDP was calculated according to Manoharan and Banerjee (1985) and Waters et al. (1990) using the formula:

$$\%R = \frac{A - B}{A - C} \times 100$$

where %*R* is the reduction percentage, *A* the micronuclei (MN) frequency after treatment with cDDP, *B* the MN frequency after treatment with the bixin and cDDP, and *C* the MN frequency of negative control.

2.5. Single-cell gel electrophoresis (SCGE, comet assay)

Comet assays were performed under alkaline conditions according to the methods of Singh et al. (1988) and Tice et al. (2000), with slight modifications. PC12 cells seeded at 1×10^6 cells in a 25 cm^2 flask. To analyze the genotoxic effect, the cells were treated with bixin or cisplatin for 4 h. And to analyze the antigenotoxic effect, the cells were treated with bixin 1 h before treatment with cisplatin. A negative control (untreated culture) and solvent control (culture of 0.4% DMSO-treated cells) were also evaluated. The total treatment time was also 4 h. Microscope slides (frosted) were covered by a thin layer of 1.0% normal-melting point agarose (Invitrogen), which was dissolved in Ca^{2+} and Mg^{2+}-free PBS, at 60 °C. Upon solidification of the agarose, 10 μL of a freshly prepared suspension of treated or control PC12 cells was mixed with 100 μL of low-melting-point agarose (0.5%, Sigma–Aldrich Co) at 37 °C. The cells were covered with a coverslip and incubated at 4 °C for 10 min. After solidification of the agarose, the coverslips were removed and the slides were incubated in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris [pH 10], 1% Triton X-100 and 10% DMSO) at 4 °C overnight. After lysis, the slides were placed in a horizontal electrophoresis unit containing alkaline electrophoretic solution (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min at 4 °C with an electric field strength of 25 V (0.78 V/cm) and a current of 300 mA, which allows the DNA to unwind, exposing alkali-labile sites. The slides were then washed with cold neutralization buffer (0.4 M Tris–HCl, pH 7.5) for 15 min. After drying at room temperature, the slides were stained with 30 μL ethidium bromide (20 μg/mL) and immediately analyzed using a fluorescence microscope (Axiostar plus, Zeiss, Germany) equipped with a 515–560 nm excitation filter, a 590 nm barrier filter and an integrated digital camera. Slides were evaluated using CometScore software (TriTek Corp., USA), and 100 randomly selected nucleoids (50 nucleoids from each replicate slide) were analyzed per treatment. Three independent experiments were performed. DNA damage was quantified by the percentage of DNA in the comet tail (% DNA).

2.6. Statistical methods

The results presented are expressed as the mean ± standard deviation of three independent experiments ($N = 3$). The data were subjected to multiple variance analysis (ANOVA) and the *post hoc* Tukey test, using GraphPad Prism 2.01 software. A value of $p < 0.05$ was considered statistically significant for all of the parameters evaluated.

3. Results

3.1. Cytotoxicity of doxorubicin and bixin in tumor cell line PC12

The citotoxicity of PC12 cells treated with bixin was evaluated by the MTT method. Cells were treated with bixin at concentrations between 0.05 and 20.0 μg/mL. Two controls were used in these experiments: a negative control (untreated cells) and a solvent control (0.4% DMSO). The negative control was considered to have a cell viability of 100%.

As shown in Fig. 1, only treatments with 0.05, 0.08 and 0.10 μg/mL bixin were moderately cytotoxic (relative MTT activity > 80%). After treatment with 0.3 μg/mL bixin, PC12 cells presented citotoxicity above 50%. The solvent control was not statistically different ($p < 0.05$) from the negative control, indicating that the solvent did not interfere with the cytotoxicity of bixin.

Using the concentrations of bixin that were moderately cytotoxic, another experiment was performed to evaluate the effects of this carotenoid on PC12 cells in combination with cisplatin (Fig. 2). When cells were treated with bixin 1 h prior to the addition of cisplatin, there was no statistically significant difference ($p > 0.05$) when compared to treatment with cisplatin alone. The concentration of cisplatin used (0.1 μg/mL) was determined in preliminary experiments (Mendonça et al., 2009). This concentration was chosen due to its low cytotoxicity (7%), sufficient BN cell numbers and ability to induce high numbers of micronuclei in PC12 cells.

Based on this information about the citotoxicity of the bixin and cisplatin in these cells, we studied the genotoxicity and antigenotoxicity of bixin (0.05, 0.08, and 0.1 μg/mL) using the micronucleus test and the comet assay.

3.2. Micronucleus test

The parameters evaluated in the micronucleus test were as follows: the percentage of binucleated cells (BN), the cytokinesis block proliferation index (CBPI) and the frequency of binucleated cells with micronuclei (MN).

Treatment with cisplatin reduced the CBPI value compared to the negative control, but this reduction was not significant ($p > 0.05$). This result reinforces the low cytotoxicity associated with this concentration of cisplatin, which produced 56% BN cells. This concentration of BN cells allowed us to perform an analysis of MN frequency. According to Fenech (2000), to analyze the frequency of MN, the percentage of BN cells should not be less than 30%. Cisplatin treatment resulted in an 8-fold increase in MN-positive cells compared with the negative control (Table 1).

These results demonstrate that the CBPI values for the different bixin treatments (0.05, 0.08, and 0.1 μg/mL) were not statistically different ($p > 0.05$) in relation to the negative control and the solvent control, and these concentrations were sufficient for BN cells to be analyzed for MN frequency (Table 1). As shown in Table 1, the number of MN in cells treated with bixin increased with the increasing concentrations evaluated (8.7, 12.0, and 18.3, respectively), but there was no significant difference ($p > 0.05$) compared to the negative control and solvent control. These results suggest that bixin is not genotoxic to PC12 cells at the concentrations evaluated.

Based on the results presented above, we evaluated the protective effects of bixin on genotoxicity induced by cisplatin in PC12 cells (Fig. 3 and Table 1).

Cells treated with bixin and cisplatin were not statistically different ($p > 0.05$) in terms of CBPI value from the negative control and cells treated with DMSO + cisplatin. All treatments had a percentage of BN cells sufficient for analysis of the number of micronuclei (Table 1). The micronucleus frequency of cells pretreated with 0.05, 0.08 and 0.10 μg/mL bixin in combination with cisplatin resulted in an 2 to 3-fold decrease significantly ($p < 0.05$) in MN-positive cells compared the positive control (DMSO + 0.1 μg/mL cisplatin). The largest reduction was observed in the pre-treatment with 0.1 μg/mL bixin (Table 1).

3.3. Comet assay

The comet assay was used to analyze the percentage of DNA in the tail; the results are presented in Fig. 3. Bixin alone had no genotoxic effect, and treatment with 0.08 and 0.1 μg/ml significantly reduced DNA migration compared with the solvent control, DMSO ($p < 0.05$). When considering genotoxicity, bixin did not induce DNA damage compared with DMSO alone.

There was no significant effect of cisplatin on DNA migration when compared with DMSO alone (Fig. 3). However, pretreatment with a combination of bixin and cisplatin caused a statistically significant decrease ($p < 0.05$) in DNA migration compared with cisplatin treatment alone.

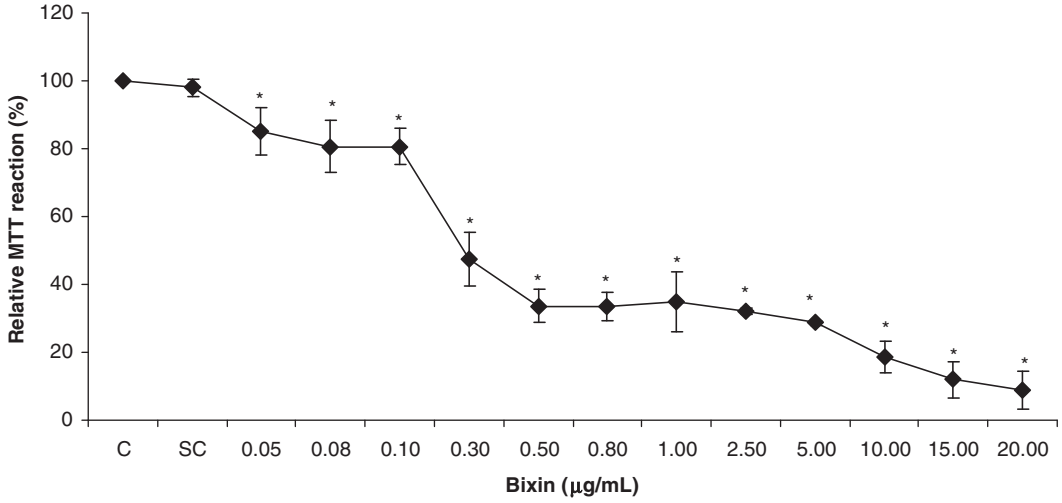

Fig. 1. Cytotoxicity of PC12 cells after treatment for 24 h with different concentrations of bixin. PC12 cell cytotoxicity was determined using the MTT assay. Values are expressed as the percentage of viable cells, with the viability of untreated control cells set at 100%. Data are mean and S.D. values from three independent experiments. *$p < 0.05$, relative to untreated cells.

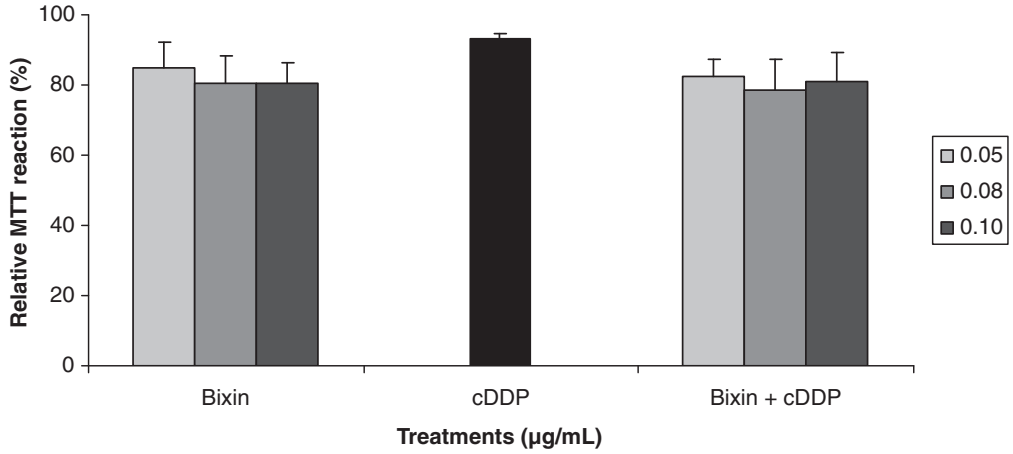

Fig. 2. Cytotoxicity of PC12 cells after treatment for 24 h with bixin, cisplatin or combined treatment with bixin and cisplatin. PC12 cell cytotoxicity was determined using the MTT assay. Data are mean and S.D. values from three independent experiments. $^{*}p < 0.05$, relative to cisplatin treatment. cDDP: cisplatin (0.1 µg/mL).

Table 1
Induction of micronuclei by bixin, cisplatin or associated treatment of bixin with cisplatin in PC12 cells.

Treatments (µg/mL)	Total cells scored	% BN	CBPI mean ± S.D.	Binucleated micronucleated cells	Binucleated micronucleated cells/1000 BN cells Mean ± S.D.	Reduction (%)
Negative control	6000	63	1.65 ± 0.17	64	10.7 ± 0.6	–
DMSO 0.4%	6000	62	1.65 ± 0.21	86	14.3 ± 1.5	–
BXN 0.05	6000	62	1.63 ± 0.15	52	8.7 ± 1.5	–
BXN 0.08	6000	59	1.61 ± 0.18	72	12.0 ± 1.0	–
BXN 0.1	6000	51	1.53 ± 0.21	109	18.3 ± 6.5	–
DMSO 0.4% + cDDP 0.1	6000	56	1.56 ± 0.11	540	92.8 ± 12.4[*]	–
BXN 0.05 + cDDP 0.1	6000	44	1.45 ± 0.30	240	40.0 ± 6.2[*,#]	67.2
BXN 0.08 + cDDP 0.1	6000	49	1.50 ± 0.29	170	28.3 ± 2.1[*,#]	82.1
BXN 0.10 + cDDP 0.1	6000	45	1.46 ± 0.30	156	26.0 ± 1.0[*,#]	85.1

Abbreviations: BN: binucleate cells; MN: micronuclei; CBPI: cytocinesis-block proliferating index; cDDP: cisplatin; BXN: bixin. Data were represented as mean of three independent experiments. Each experiment was done in triplicate.
[*] Statistically significant difference in comparison with Negative control and DMSO ($p < 0.05$).
[#] Statistically significant difference in comparison with DMSO + cDDP ($p < 0.05$).

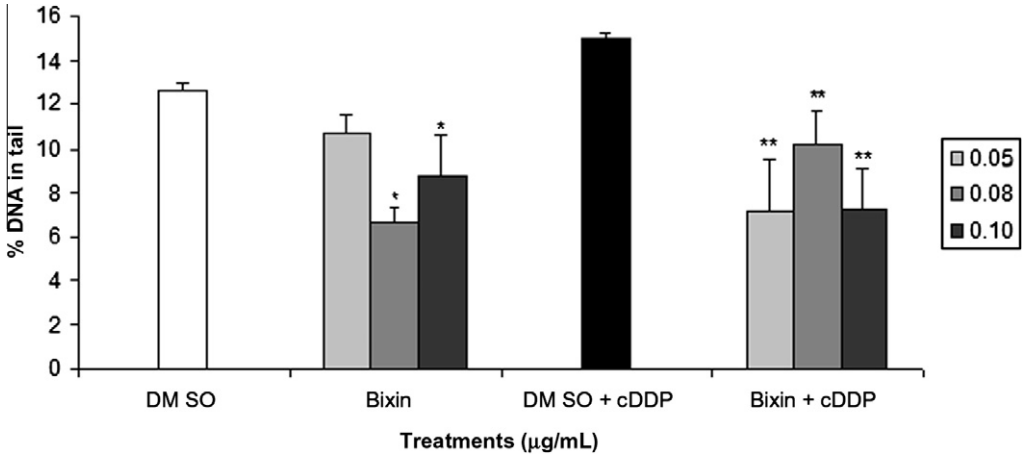

Fig. 3. Percentage of DNA damage in PC12 cells treated for 4 h with bixin, cisplatin or combined treatment with bixin and cisplatin. $^{*}p < 0.05$, relative to DMSO treatment and $^{**}p < 0.05$, relative to DMSO + cDDP treatment. cDDP: cisplatin.

4. Discussion

The mechanism by which cisplatin induces neurotoxicity is still unclear. This peripheral neuropathy may be due in part to oxidative stress (Lee et al., 2008). Thus, an antioxidant able to neutralize or reduce the action of ROS during treatment with cisplatin may reduce the drug's toxic effects on normal cells. The carotenoid bixin may contribute to protecting these cells against the deleterious

effects of free radicals (Di Mascio et al., 1990), as it is an effective scavenger of singlet oxygen molecules, and it has the potential to act specifically on DNA damage induced by free radicals or other compounds capable of generating such damage (Rios et al., 2009). Leonetti et al. (2003) suggested that antioxidants do not interfere with the antitumor activity of cisplatin and that they are safe to use during chemotherapy. They found that alpha-tocopherol protected against the systemic toxicity and neurotoxicity induced by cisplatin without interfering with antitumor activity in both *in vitro* and *in vivo* systems.

Although widely used as a food-coloring agent with antioxidant activity, there are still only a few, limited studies on the biological effects of bixin.

All concentrations of bixin tested inhibited PC12 tumor cell growth, as indicated by the MTT assay, although 0.05, 0.08, and 0.1 μg/mL were only moderately cytotoxic (relative MTT activity > 80%).

Reddy et al. (2005) evaluated the cell growth inhibition induced by various naturally colored foods, including bixin, in the tumor cell lines HCT-116 (colon), AGS (stomach), SF-268 (CNS), and NCI-H460 (lung). Bixin had an IC_{50} of 33, 49, 45, and 39 μg/mL in cell lines from colon cancer, the central nervous system, stomach and lung, respectively. Tibodeau et al. (2010) also observed the cytotoxic effects of bixin in a variety of cancer cell lines such as U2OS (osteosarcoma), PC3 (prostate), HCT-116 (colon), MCF7 (breast), DRO (anaplastic thyroid), and BHP5–16 (papillary thyroid), and obtained IC_{50} values of 4–20 μg/mL after 24 h of exposure. This group also demonstrated that bixin rapidly induced ROS in a dose- and time-dependent manner based on fluorescence activated cell sorting (FACS) experiments, suggesting that the ROS generation induced by bixin contributes to its cytotoxicity. Like other dietary phytochemicals, bixin has pro-oxidant activity in addition to its antioxidant activity, depending on the dosage and chemical environment. Thus, depending on the concentration used, bixin can act as a pro-oxidant, causing cell death.

When PC12 cells were pretreated with a combination of cDDP and bixin, we observed that this combination did not change the individual cytotoxicity profiles of these compounds, indicating that bixin does not increase the cytotoxicity of cDDP under the evaluated conditions.

According to Zeiger (2007), for a substance to be classified as antigenotoxic/antimutagenic, it must first be evaluated for genotoxicity/mutagenicity. In our study, the three concentrations of bixin (0.05, 0.08, and 0.1 μg/mL) that maintained cell viability above 80% were evaluated using the comet assay and the micronucleus test to determine the compound's genotoxic potential prior to assessing it as a potential protective agent.

In the micronucleus test, the concentrations of bixin assayed did not produce effects that were statistically different ($p > 0.05$) from the solvent control. In the comet assay, bixin did not induce DNA damage in PC12 cells but actually reduced damage when compared with the baseline solvent control, as shown by the decreased DNA fragmentation in bixin-treated cells. These results indicate that under our experimental conditions, bixin is not genotoxic to PC12 cells.

With respect to cDDP, our comet assay results indicated that this drug did not significantly affect the migration of DNA when compared with the DMSO control. This migration is inhibited by interstrand DNA crosslinks and facilitated by intrachain crosslinks (Tice et al., 2000; Wozniak et al., 2004). Thus, the existence of interstrand crosslinks inhibits the electrophoretic mobility of DNA, reducing the amount of DNA found in tails (Almeida et al., 2006). Wozniak et al. (2004) demonstrated that cDDP can induce DNA fragmentation and can also cause crosslinking in DNA from human lymphocytes and K562 cells (human chronic myelogenous leukemia).

Overall, we observed potential protective effects associated with bixin. This conclusion is based on the results of the micronucleus test, which showed a difference in the frequency of micronuclei in cells treated with bixin and cDDP compared to DMSO + cDDP treatment. Treatment with cDDP and bixin did not decrease the frequency of micronuclei to the values observed in the negative control ($p < 0.05$), but the values were significantly decreased ($p < 0.05$) when compared with those for cells treated with DMSO + cDDP. Similarly, other studies have shown that bixin has a protective effect against radiation damage and against cDDP-induced clastogenicity in rats (Silva et al., 2001; Thresiamma et al., 1998).

The mechanisms by which carotenoids induce genotoxicity and/or antigenotoxicity are not well understood. The antioxidant mechanism *in vitro* might involve the direct inhibition of ROS generation or sequestering of free radicals (Aruoma, 2003). Rios et al. (2009) assessed the modulation of ROS produced by the interaction of cDDP and DNA in a human cell-free experimental model using bixin. The results revealed that the ROS generated by the cDDP/DNA interaction were inhibited by 40 μg/mL of bixin (superoxide anion generation at 82% and total ROS generation at 42%).

Because bixin is used as a natural colorant in various foods, its bioactivity and toxicity have been studied previously, including its effect on lipid peroxidation and carcinogenesis (Kiokias and Gordon, 2003; Reddy et al., 2005; Silva et al., 2001). Additionally, it is thought that bixin suppresses the formation and proliferation of tumors. This effect is probably due to both its antioxidant effects and its inhibition of DNA breaks and lipid peroxidation.

5. Conclusion

The antioxidant effects produced by the ability of bixin to intercept free radicals generated by cDDP may have contributed, at least partially, to the antigenotoxic effects observed in this study. However, more studies are being conducted to better understand the mechanisms underlying the protective activity of this carotenoid.

6. Conflict of Interest

The authors declare that there are no conflicts of interest.

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