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Cytotoxic effects of *Vitis labrusca* (fox grape) whole juices on human tumor and non-tumor cells, *in vitro*

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ABSTRACT. Cancer is a public health problem of increasing concern worldwide due to the growing increase in mortality rates. The identification of natural compounds with cytotoxic activity is important due to the ready availability and their ability to act effectively in reducing the incidence of cancer, without adverse side effects. Whole grape juice (*Vitis labrusca*) is often consumed by the population, which is an example of a product with a high content of bioactive compounds, such as flavonoids and resveratrol. To help determine the suitability of this natural product as a health aid, we evaluated the cytotoxic activity of organic and conventional whole grape juices, exposed or not to UV-C irradiation, on human liver tumor cells (HepG2/C3A) and on non-tumor lung cells (MRC-5), by the MTT assay (a colorimetric assay for assessing cell metabolic activity), for 24 and 48 h. All of the juices, at the highest concentrations (50-100 µL/mL), showed significant cytotoxic activity on HepG2/C3A cells, reducing cell proliferation from 78 to 47%, in relation to the control, especially, after 48 h of treatment. Both conventional grape juices had cytotoxic effects on MRC-5

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cells at 24 h of treatment, but cell proliferation was reestablished after 48 h. Furthermore, organic grape juice stimulated the proliferation of this cell line, especially that produced from grape exposed to UV-C, possibly in consequence of the increased amount of antioxidants, such as anthocyanins, resveratrol and flavonoids in the fruits, which probably stimulated cell metabolism. This data supports the use of whole grape juice, due to it's nutraceutical potential, mainly because of antioxidant activity, in particular organic juice, and our findings demonstrate the benefit of food production techniques that add nutritional value, such as post-harvest exposure to UV-C.

Key words: Anti-tumor Effects; Conventional Production Systems; Natural Organic Food; UV-C Irradiation; Whole Grape Juice

INTRODUCTION

Cancer is a worldwide distributed disease that increasingly affects the human population with different backgrounds and induction factors. According to the medical diagnosis, which depends on the type and the location of the tumor, cancer may be treated with chemotherapy, employing the use of one or more chemical drugs, and/or by radiation therapy, associated or not with surgical procedures.

The use of radiopharmaceuticals for the diagnosis and the treatment of tumors, as well as conventional chemotherapy and radiotherapy, can cause severe side effects in patients. Therefore, the identification of natural compounds with moderate cytotoxic activity is important, due to their easy availability, their low cost, and their ability to act effectively in reducing the incidence of cancer, without adverse side effects (Serra et al., 2013). In this sense, fox grapes (*Vitis labrusca*) and their derivatives stand out, because of their several nutritional, pharmacological and therapeutic benefits, such as in the treatment of cardiovascular diseases (Quiñones et al., 2013; Huang et al., 2015), liver and kidney disorders (Ulusoy et al., 2012; Charradi et al., 2013; Kim et al., 2013), and anti-tumor activity (Zheng et al., 2012; Zhang et al., 2013; Nivelle et al., 2017), especially due to the presence of phenolic compounds and their antioxidant activity (Fernandes et al., 2013; Maestre et al., 2013; Toaldo et al., 2015; Copetti et al., 2018).

Studies have shown that the synthesis of phenolic compounds in the grapes can be increased with an exposure of the fruits to ultraviolet radiation (UV). The UV light type C (UV-C) produces an abiotic stress in the plant tissues that up-regulates the expression of specific genes involved in the growth of the cells and in the secondary metabolism in the plants, such as in the production of anthocyanins, resveratrol, flavonoids and aromatic compounds (Zhang et al., 2012; Peinado et al., 2013).

Thus, the objective of this study was to evaluate the cytotoxic and the antiproliferative activity of organic and conventional whole fox grape juices, exposed or not to UV-C irradiation, in human hepatoma cells (HepG2/C3A) and human lung fibroblasts (MRC-5), *in vitro*.

MATERIAL AND METHODS

Cell cultures

Human hepatoma cells HepG2/C3A (Cat. #0291) and the non-tumor human lung fibroblasts MRC-5 (Cat. #0180) were obtained from the Rio de Janeiro Cell Bank, Brazil. The

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cell lines were grown in 25 cm^2 culture flasks containing a complete culture medium (supplemented with 10% of fetal bovine serum and 1 mL/L of antibiotic/antimycotic solution) and maintained in a CO_2 incubator (5%) with 95% humidity at 37°C. For the experiments, the cells were used in *log* phase.

Treatment solutions

Fox grapes, *V. labrusca* of the Concord variety, from the 2012 harvest, were produced in the municipality of Verê, belonging to a micro-region of Francisco Beltrão, Paraná – Brazil. The grapes were obtained from two nearby properties (5.3 km apart), one using conventional farming practice (564 m altitude, latitude 25º 54' 01" S and longitude 52º 53' 51" W) and the other one with a practice of organic farming (492 m altitude, latitude 25º 51' 21" S and longitude 52º 55' 06" W), under similar climatic and edaphology conditions.

The grapes were selected according to their appearance, washed in chlorinated water (sodium hypochlorite, 50 mg/L, pH 5.0) and stored under refrigeration (± 5°C). One part of the grapes that were collected from each culture system was subjected to a post-harvest treatment with UV-C irradiation, according to the method as described by Cantos et al. (2001), with modifications. The following parameters were used: a radiation rate of 65.6 J/m^2 at a distance of 30 cm from the light source. The grapes were arranged in a single layer on trays, which were irradiated in a chamber equipped with three UV-C lamps (Philips® 90 W) for 5 min. After that, the grapes were rotated vertically 180° and the light source was maintained for another 5 min, totaling 10 min of irradiation. The irradiated fruits were stored for three days at 25 ± 5°C in the absence of light, to promote the biosynthesis of the bioactive compounds.

The conventional and organic grapes, with or without a post-harvest treatment with UV-C, were used to produce whole juices that were obtained by a steam distillation extractor at 90°C and were stored in glass bottles. Sterile samples of organic, conventional, organic UV-C treated, and conventional UV-C treated juices, were stored at -4ºC and were defrosted at the time of the experimentation.

Chemical agents

Methyl methanesulfonate (MMS - 99%) and dimethyl sulfoxide (DMSO - 99.7%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), Antibiotic/Antimycotic and MTT (3-[4,5-dimethilthiazol-2-yl]-2,5-dipheniltetrazolium bromide, 98%) were purchased from Gibco® Life Technologies (Carlsbad, CA, USA).

MTT cytotoxicity assay

The cytotoxicity test was run based on the ability of cellular and mitochondrial dehydrogenase enzymes of viable cells to convert MTT into formazan, according to the protocols suggested by Mosmann (1983), with modifications. The cells (HepG2/C3A or MRC-5) were seeded in 96-well culture plates at a density of 10^4 cells per well, except for the control wells that were without cells (blank). After 24 h of stabilization, the culture medium was discarded and 100 µL of fresh complete medium for the groups: control (CO), cytotoxic agent MMS (50 µM), and different concentrations of organic, conventional, organic post-treated UV-C, and conventional post-treated UV-C whole juices (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µL/mL).

After 24 or 48 h of incubation, the culture medium was replaced by 100 µL of fresh culture medium supplemented with MTT (0.2 mg/mL). The plates were incubated for a further 4 h before discarding the medium containing the MTT, followed by the addition of 100 µL of DMSO for the formazan crystal solubilization. The absorbance measurement was performed in a microplate reader (Labtech) at 550 nm. The cell proliferation was estimated based on the absorbance of the control by the following formula: $A_{treatment}/A_{control}$ *x 100*. The experiments were performed by three independent repetitions.

Statistical analyzes

The results were presented as mean and standard deviations and were subjected to an analysis of variance (one way ANOVA) followed by Dunnett's test using the GrafPad® Prism 5 software. The differences were considered statistically significant when the P value was less than 0.05.

RESULTS AND DISCUSSION

The results of the cytotoxicity assay when using different concentrations of the organic whole grape juice (Figure 1-A) showed that all of the concentrations starting at 20 µL/mL of the juice were cytotoxic to human liver tumor cells (HepG2/C3A) in a dose dependent manner after 48 h of treatment. Only the concentration of 40 µL/mL showed cytotoxic effect after 24 h of treatment. On the other hand, the data in Figure 1-B showed that none of the concentrations of the organic grape juice was cytotoxic to non-tumor human lung cells (MRC-5) after 24 and 48 h of treatment. Furthermore, all of the treatments at both treatment periods maintained the cell proliferation of the MRC-5 cells above 90% and the concentrations above 30 µL/mL at 48 h significantly reduced the proliferation of HepG2/C3A cells at 54% (100 µL/mL), in relation to the control (100%, Table 1). For both cell lines, only the MMS presented a cytotoxic effect at 24 and 48 h, significantly reducing cell proliferation to below 80%.

Similar to our study, Hakimuddin et al. (2006) showed that polyphenolic compounds of red wine, including aglycone flavonoids, presented a maximal growth inhibition of breast cancer cells (MCF-7) with a low cytotoxicity on the normal human mammary epithelial cells (HMEC) and the normal immortalized breast cells (MCF-10A). The absence of toxicity on the non-tumor cells was confirmed by Charles et al. (2002) when testing green grapes in Chinese hamster ovary cells (CHO-K1-BH4). The antitumor activity was confirmed by grape peel extracts on mice breast tumor cells (4T1) (Sun et al., 2012); by grape seed extracts on prostate carcinoma cells (PC3) (Ignea et al., 2013); and by proanthocyanidins (flavonoids presented in grapes) on murine lymphosarcoma cells (YAC-1), lung carcinoma (SPC-A-1) and human lymphoma (K-562) (Zhang et al., 2005).

Resveratrol (3,4',5-trihydroxy-*trans*-stilbene), a natural polyphenol present in grapes, has been shown to inhibit intestinal tumorigenesis and modulate host-defense-related gene expression in an animal model of human familial adenomatous polyposis (Schneider et al., 2001), in addition to exerting anticancer effects on HepG2 cells (Zheng et al., 2012; Scherzberg et al., 2015), on human nasopharyngeal carcinoma (NPC) (Zhang et al., 2013), colon carcinoma (HT-29) and colorectal carcinoma (CaCo-2) (Scherzberg et al., 2015). Chen et al. (2004) reported that resveratrol caused significant cytotoxicity and increased apoptosis and S-phase accumulation of neuroblastoma cells due to the down-regulation of p21 and up-regulation of cyclin E. Resveratrol analogs were efficient in inducing the accumulation of human colon cancer cells in early S-phase of the cell cycle. This effect was associated with a nuclear redistribution of

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cyclin A and the formation of a cyclin A/cyclin-dependent kinase 2 complex whose kinase activity was increased (Colin et al., 2009). According to Pinto (2013), resveratrol was found in the grape juices of this study and may have contributed to these results.

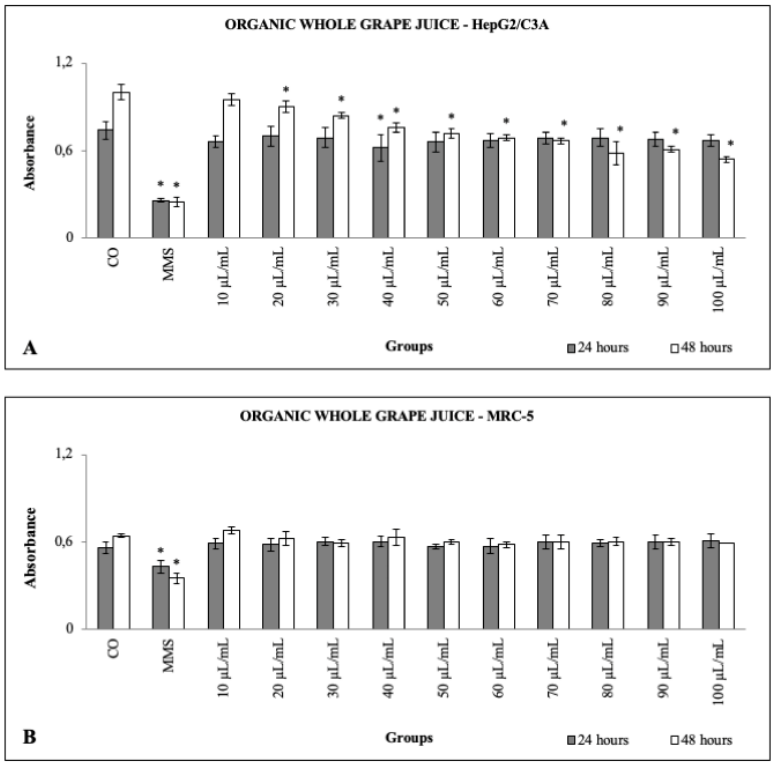

Figure 1: Mean absorbance and standard deviation as an indication of HepG2/C3A (A) and MRC-5 (B) cell proliferation, after treatment with different concentrations of the organic whole grape juices. CO: Control; MMS: methyl methanesulfonate (50 µM); 10^4 cells per well, incubated for 24 and 48 h, *n*=3. * Different from Control ($P < 0.05$, Dunnett's test).

Table 1. Cell proliferation estimated based on the absorbance of HepG2/C3A and MRC-5 cells treated with organic grape juices, exposed or not to UV-C irradiation, for 24 and 48 h.

Groups		CO	MMS	10 µL/mL	20 µL/mL	30 µL/mL	40 µL/mL	50 µL/mL	60 µL/mL	70 µL/mL	80 µL/mL	90 µL/mL	100 µL/mL
Organic grape juice													
HepG2/C3A (%)	24 h	100.0	35.1	89.1	94.5	93.2	83.7	89.1	90.5	93.2	93.2	91.8	90.5
	48 h	100.0	25.0	95.0	90.0	84.0	76.0	72.0	69.0	67.0	58.0	61.0	54.0
MRC-5	24 h	100.0	76.7	105.3	103.5	107.1	107.1	101.7	101.7	107.1	105.3	107.1	108.9
(%)	48 h	100.0	54.6	106.2	96.8	92.1	98.4	93.7	90.6	93.7	93.7	93.7	92.1
UV-C organic grape juice													
HepG2/C3A (%)	24 h	100.0	65.1	99.0	93.5	87.1	81.6	80.7	80.7	79.8	78.0	76.1	70.6
	48 h	100.0	71.2	105.6	101.6	94.4	84.0	76.0	68.8	61.6	54.4	48.8	47.2
MRC-5	24 h	100.0	57.2	103.5	103.9	110.3	111.0	115.0	118.1	114.1	114.1	115.3	117.6
(%)	48 h	100.0	35.8	101.1	96.7	95.9	97.2	100.4	100.4	100.2	100.4	97.7	99.1

Groups: CO: Control; MMS: methyl methanesulfonate (50 µM), different concentrations of grape juices exposed or not to UV-C; 10^4 cells per well, incubated for 24 and 48 h, *n*=3.

For the organic grape juice that was exposed to UV-C (Figure 2-A), the cytotoxic/antitumor effect was more evident for concentrations starting at 20 µL/mL (at 24 h) and 30 µL/mL (at 48 h) for the HepG2/C3A metabolizing cells. For some of these groups, the cell proliferation was less than 80% at 24 h for those concentrations from 70 to 100 µL/mL and at 48 h for those concentrations from 50 to 100 µL/mL, compared to the control (100%, Table 1), both having a dose-dependent cytotoxic effect, as the increment of juice concentration resulted in a greater cytotoxicity. At 48 h, it was observed that the concentrations required to inhibit 50% of the tumor cell proliferation was between 90 and 100 µL/mL for the organic grape juice that was treated with UV-C.

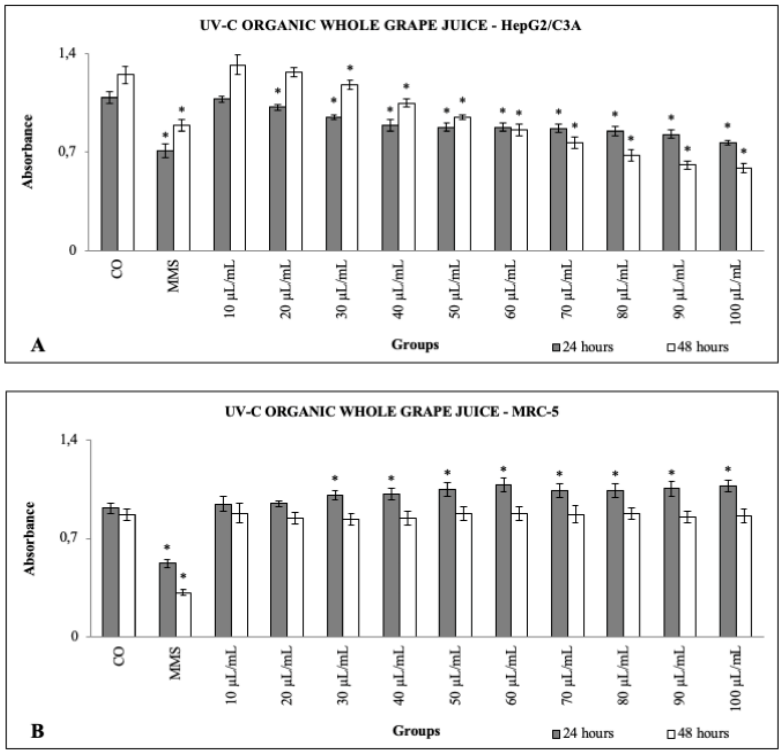

Figure 2: Mean absorbance and standard deviation as an indication of HepG2/C3A (A) and MRC-5 (B) cell proliferation, after treatment with different concentrations of the UV-C organic whole grape juices. CO: Control; MMS: methyl methanesulfonate (50 µM); 10^4 cells per well, incubated for 24 and 48 h, *n*=3. * Different from Control ($P < 0.05$, Dunnett's test).

On the other hand, when this juice was administered to the non-metabolizing non-tumor cells (MRC-5) (Figure 2-B), all of the concentrations starting at 30 µL/mL at 24 h, instead of inhibiting the cell proliferation as was observed in the tumor cells, increased cellular metabolism, resulting in a significant increment in absorbance and cell viability, when compared to the control (Table 1). However, at 48 h, this metabolic stimulation was stabilized, resulting in proliferation similar to the control. Only the cytotoxic agent MMS, at 24 and 48 h, was cytotoxic to the HepG2/C3A and MRC-5 cells, reducing cell proliferation (less than 71.2%) when compared to the control.

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The treatment of the grapes with the UV-C irradiation increased the biosynthesis of the phenolic compounds, such as the resveratrol and chalcone derivatives (flavonoids, anthocyanins and the aromatic compounds) (Tang et al., 2010; Peinado et al., 2013). The physicochemical analyzes of the juices in the present study showed that the levels of cyanidin, epicatechin and *cis*-resveratrol was increased in the UV-C-treated organic grape juices (data published by Pinto 2013). These compounds might have been responsible for the cellular metabolism stimulation of the non-tumor lung cells, as observed by Sebastià et al. (2013), who found a stimulation of the proliferation in human peripheral lymphocytes after the administration of resveratrol. The hepatocellular carcinoma cells did not respond similarly to the effect of the UV-C-treated whole juices, as the cell proliferation decreased due to cytotoxicity.

The conventional whole grape juices were cytotoxic for the HepG2/C3A cells at concentrations above 30 µL/mL, at 24 and 48 h, based on Dunnett's test (Figure 3-A), with a cell proliferation below 80%, when compared to the control (100%), at 48 h for those concentrations ranging from 70 to 100 µL/mL (Table 2), with a dose-dependent effect. The MMS treatment was different from the control at 24 and 48 h, with cell viability below 80%. However, the conventional grape juices were cytotoxic to the MRC-5 cells at concentrations ranging from 10 to 70 µL/mL at 24 h (Figure 3-B), with cell proliferation below 67% (Table 2). Nevertheless, this cytotoxic effect did not occur at 48 h, because the cells proliferated and exhibited absorbance similar to the control, and at this time, only the MMS was different from the control, showing a cytotoxic effect.

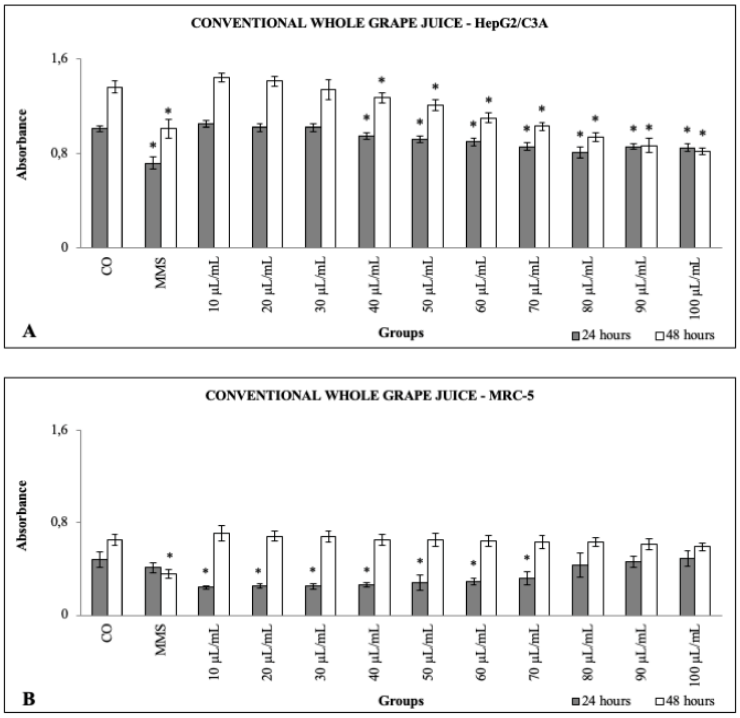

Figure 3: Mean absorbance and standard deviation as an indication of HepG2/C3A (A) and MRC-5 (B) cell proliferation, after treatment with different concentrations of the conventional whole grape juices. CO: Control; MMS: methyl methanesulfonate (50 µM); 10^4 cells per well, incubated for 24 and 48 h, *n*=3. * Different from Control ($P < 0.05$, Dunnett's test).

Table 2 – Cell proliferation estimated based on the absorbance of HepG2/C3A and MRC-5 cells treated with conventional grape juices, exposed or not to UV-C irradiation, for 24 and 48 h.

Groups		CO	MMS	10 µL/mL	20 µL/mL	30 µL/mL	40 µL/mL	50 µL/mL	60 µL/mL	70 µL/mL	80 µL/mL	90 µL/mL	100 µL/mL
Conventional grape juice													
HepG2/C3A	24 h	100.0	71.2	103.9	100.9	100.9	94.0	91.0	89.1	85.1	80.1	85.1	84.1
(%)	48 h	100.0	74.2	105.8	103.6	103.6	93.3	88.9	80.8	75.7	69.1	63.9	60.2
MRC-5	24 h	100.0	85.4	50.0	52.0	52.0	54.1	58.3	60.4	66.6	89.5	95.8	102.0
(%)	48 h	100.0	55.3	109.2	104.6	104.6	100.0	100.0	98.4	96.9	96.9	93.8	90.7
UV-C conventional grape juice													
HepG2/C3A	24 h	100.0	36.0	102.6	106.6	109.3	102.6	101.3	96.0	90.6	93.3	93.3	94.6
(%)	48 h	100.0	18.5	73.1	87.6	87.6	81.4	78.3	74.2	73.1	72.1	73.1	68.0
MRC-5	24 h	100.0	42.7	70.6	74.0	79.8	95.6	104.4	111.4	114.4	112.4	110.8	110.8
(%)	48 h	100.0	43.9	97.7	87.5	90.5	89.0	91.9	92.3	96.7	95.8	92.9	98.4

Groups: CO: Control; MMS: methyl methanesulfonate (50 µM), different concentrations of grape juices exposed or not to UV-C; 10^4 cells per well, incubated for 24 and 48 h, *n*=3.

The variations in the cytotoxic activity of the non-tumor cells on the evaluated concentrations were also shown by Jacob et al. (2008), where fractions of 60% of the grape polyphenols showed a cytotoxic activity for MCF-10A cells. Furthermore, fractions of 40% of the grape polyphenols did not inhibit the growth of the same cells, but they were effective in inhibiting the proliferation of the MCF-7 tumor cells. This could happen, as noted by Chedea et al. (2010), because the grape polyphenols have a pro-oxidant activity, depending on the dose, the exposition time, and the other dietary components.

The cytotoxic effects of the conventional grape juices were not found by Düsman et al. (2014, 2017) who evaluated the same whole juices of the present study (Düsman et al., 2014) and the juices produces from the harvest of 2011 (Düsman et al., 2017) in *Rattus norvegicus* hepatoma cells (HTC). However, Düsman et al. (2014) noted that the conventional whole grape juices that were treated or not with UV-C, induced the smallest cytokinesis-block proliferation index, as well the highest average number of micronuclei.

According to Charles et al. (2002), different agricultural practices could affect the types and the levels of toxic compounds in food. Dani et al. (2007), for example, showed that organic and conventional grape extracts were able to prevent the oxidative damage to lipids and proteins in brain tissue and that the organic extracts were more effective. Toaldo et al. (2015) showed that acute consumption of red grape (*V. labrusca*) juice produced in southern Brazil, promoted a significant decrease in lipid peroxides in serum and thiobarbituric acid reactive substances levels in the plasma of healthy subjects, probably due to the presence of polyphenols in this beverage. Similarly, in this study, the organic grape juices showed an antitumor effect at lower concentrations with no cytotoxic effects on the non-tumor cells, while the conventional grape juices showed antitumor effects and cytotoxic activity for the non-tumor cells at 24 h of treatment.

For the HepG2/C3A tumor cells (Figure 4-A), all of the tested concentrations of the UV-C-treated conventional grape juices were cytotoxic after 48 h of treatment and only the concentrations of 70 µL/mL showed this effect after 24 h of treatment. Cell proliferation (Table 2) was below 80% for the concentrations of 10, 50 to 100 µL/mL, when compared to the control (100%), after 48 h. The MMS showed cytotoxic effects at 24 and 48 h.

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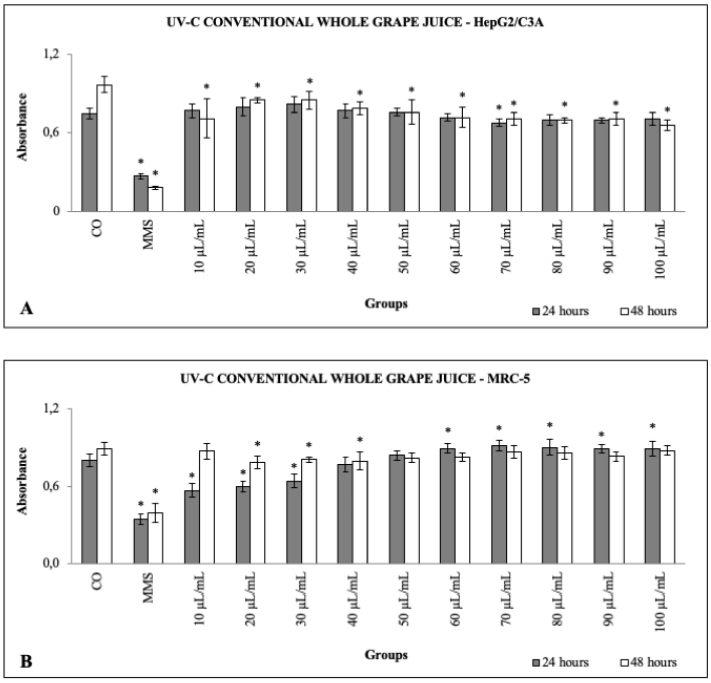

Figure 4: Mean absorbance and standard deviation as an indication of HepG2/C3A (A) and MRC-5 (B) cell proliferation, after treatment with different concentrations of the UV-C-treated conventional whole grape juices. CO: Control; MMS: methyl methanesulfonate (50 µM); 10^4 cells per well, incubated for 24 and 48 h, *n*=3. * Different from Control ($P < 0.05$, Dunnett's test).

The lower concentrations of the UV-C-treated conventional grape juices (Figure 4-B) at 24 h (10, 20 and 30 µL/mL) and at 48 h (20, 30 and 40 µL/mL) reduced the MRC-5 cell proliferation (below 80%) at 24 h (concentrations of 10, 20 and 30 µL/mL), when compared to the control (100%). However, higher concentrations (60 to 100 µL/mL) of this juice at 24 h probably stimulated the metabolism and cell proliferation of the MRC-5 cells, similar to that that was observed for the UV-C-treated organic grape juices in these cells (Figure 2-B). Moreover, as noticed for the UV-C-treated organic grape juices at 48 h, the metabolic stimulation had stabilized and resulted in equal absorbances when compared to control. This was probably due to the greater content of total phenolics and total anthocyanins, such as malvidin, delphinidin, peonidin and cyanidin from this juice (data published by Pinto 2013), whose production was stimulated by the UV-C exposition of conventional grapes, when compared to the juices that were not exposed to irradiation.

When considering the diversity of the antioxidants that are present in whole grape juices, especially those that have been exposed to UV-C irradiation, it is likely that these compounds exert their antitumor activity by an activation of multiple cellular events that are associated with the initiation, the promotion, and the progression of tumors (Zhou and Raffoul, 2012). Such mechanisms might be related to reactive oxygen species scavenging, cell cycle progression, and the regulation of pro-apoptotic and anti-apoptotic genes (Sun et al., 2011; Lin et al., 2012). As described in this study, the cytotoxicity that was observed for

the tumor cell line and the increased cell proliferation in the non-tumor cell line might be related to the mechanisms of action of these polyphenols.

Thus, the data has shown that conventional and organic whole grape juices that have been exposed or not to UV-C irradiation, presented an antitumor activity for the HepG2/C3A cells, without any deleterious effects on the non-tumor cells, stimulating their metabolism after 24 h of treatment, especially for those higher concentrations that were evaluated. The results of this study arouse the interest for further research on the potential of whole grape juices, suggesting their use as an adjuvant to those patients undergoing chemotherapy or radiotherapy treatment. The benefits might be associated with a cytotoxic activity on tumor cells; a protection for non-tumor cells; an aid in the recovery of patients undergoing conventional cancer therapy; and all of this is besides the nutraceutical benefits that are provided by these whole grape juices.

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REFERENCES

Cantos E, Espín JC and Tomás-Barberán FA (2001). Postharvest induction modeling method using UV irradiation pulses for obtaining resveratrol-enriched table grapes: a new functional fruit? *J. Agric. Food Chem.* 49: 5052–8.

Charles GD, Linscombe VA, Tornesi B, Mattsson JL, et al. (2002). An *in vitro* screening paradigm for extracts of whole foods for detection of potential toxicants. *Food Chem. Toxicol.* 40: 1391–1402.

Charradi K, Elkahoui S, Karkouch I, Limam F, et al. (2013). Grape seed and skin extract alleviates high-fat diet-induced renal lipotoxicity and prevents copper depletion in rat. *Appl. Physiol. Nutr. Metab.* 38: 259–267.

Chedea VS, Braicu C and Socaciu C (2010). Antioxidant/prooxidant activity of a polyphenolic grape seed extract. *Food Chem.* 121: 132–139.

Chen Y, Tseng SH, Lai HS and Chen WJ (2004). Resveratrol-induced cellular apoptosis and cell cycle arrest in neuroblastoma cells and antitumor effects on neuroblastoma in mice. *Surgery*. 136: 57–66.

Colin D, Gimazane A, Lizard G, Izard JC, et al. (2009). Effects of resveratrol analogs on cell cycle progression, cell cycle associated proteins and 5fluoro-uracil sensitivity in human derived colon cancer cells. *Int. J. Cancer*. 124: 2780–2788.

Copetti C, Franco FW, Machado ER, Soquetta MB, et al. (2018). Acute consumption of bordo grape juice and wine improves serum antioxidant status in healthy individuals and inhibits reactive oxygen species production in human neuron-like cells. *J. Nutr. Metab.* 2018: 1-11.

Dani C, Oliboni LS, Vanderlinde R, Bonatto D, et al. (2007). Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. *Food Chem. Toxicol.* 45: 2574–2580.

Düsman E, Almeida IV, Pinto EP, Lucchetta L, et al. (2017). Influence of processing and storage of integral grape juice (*Vitis labrusca* L.) on its physical and chemical characteristics, cytotoxicity, and mutagenicity in vitro. *Genet. Mol. Res.* 16: 1-12.

Düsman E, De Almeida IV, Lucchetta L and Vicentini VEP (2014). Effect of processing, post-harvest irradiation, and production system on the cytotoxicity and mutagenicity of *Vitis labrusca* L. juices in HTC cells. *PLoS One* 9: 1-6.

Fernandes F, Ramalhosa E, Pires P, Verdial J, et al. (2013). *Vitis vinifera* leaves towards bioactivity. *Ind. Crops Prod.* 43: 434–440.

Hakimuddin F, Paliyath G and Meckling K (2006). Treatment of MCF-7 breast cancer cells with a red grape wine polyphenol fraction results in disruption of calcium homeostasis and cell cycle arrest causing selective cytotoxicity. *J. Agric. Food Chem.* 54: 7912–7923.

Huang LL, Pan C, Wang L, Ding L, et al. (2015). Protective effects of grape seed proanthocyanidins on cardiovascular remodeling in DOCA-salt hypertension rats. *J. Nutr. Biochem.* 26: 841–849.

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Ignea C, Dorobanţu CM, Mintoff CP, Branza-Nichita N, et al. (2013). Modulation of the antioxidant/pro-oxidant balance, cytotoxicity and antiviral actions of grape seed extracts. *Food Chem.* 141: 3967–3976.

Jacob JK, Hakimuddin F, Paliyath G and Fisher H (2008). Antioxidant and antiproliferative activity of polyphenols in novel high-polyphenol grape lines. *Food Res. Int.* 41: 419–428.

Kim Y, Choi Y, Ham H, Jeong HS, et al. (2013). Protective effects of oligomeric and polymeric procyanidin fractions from defatted grape seeds on tert-butyl hydroperoxide-induced oxidative damage in HepG2 cells. *Food Chem.* 137: 136–141.

Lin YS, Chen SF, Liu CL and Nieh S (2012). The chemoadjuvant potential of grape seed procyanidins on p53-related cell death in oral cancer cells. *J. Oral Pathol. Med.* 41: 322–331.

Maestre R, Douglass JD, Kodukula S, Medina I, et al. (2013). Alterations in the intestinal assimilation of oxidized PUFAs are ameliorated by a polyphenol-rich grape seed extract in an *in vitro* model and CaCo-2 cells. *J. Nutr.* 143: 295–301.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* 65: 55–63.

Nivelle L, Hubert J, Courot E, Borie N, et al. (2017). Cytotoxicity of labruscol, a new resveratrol dimer produced by grapevine cell suspensions, on human skin melanoma cancer cell line HT-144. *Molecules*. 22: 1–11.

Peinado J, Lerma NL, Peralbo-Molina A, Priego-Capote F, et al. (2013). Sunlight exposure increases the phenolic content in postharvested white grapes. An evaluation of their antioxidant activity in Saccharomyces cerevisiae. *J. Funct. Foods.* 5: 1566–1575.

Pinto EP (2013). Sistema de produção e radiação UV-C na síntese de compostos bioativos em uvas (*Vitis labrusca*, cv. Concord) e seus sucos. Doctoral Dissertation. Universidade Federal de Pelotas, Pelotas. Available at [http://dctaufpel.com.br/ppgcta/manager/uploads/documentos/teses/tese_pinto,_ellen_porto_2013.pdf].

Quiñones M, Guerrero L, Suarez M, Pons Z, et al. (2013). Low-molecular procyanidin rich grape seed extract exerts antihypertensive effect in males spontaneously hypertensive rats. *Food Res. Int.* 51: 587–595.

Scherzberg MC, Kiehl A, Zivkovic A, Stark H, et al. (2015). Structural modification of resveratrol leads to increased anti-tumor activity, but causes profound changes in the mode of action. *Toxicol. Appl. Pharmacol.* 287: 67–76.

Schneider Y, Duranton B, Gossé F, Schleiffer R, et al. (2001). Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. *Nutr. Cancer*. 39: 102–107.

Sebastià N, Almonacid M, Villaescusa JI, Cervera J, et al. (2013). Radioprotective activity and cytogenetic effect of resveratrol in human lymphocytes: An *in vitro* evaluation. *Food Chem. Toxicol.* 51: 391–395.

Serra A, Bladé C, Arola L, Macià A, et al. (2013). Flavanol metabolites distribute in visceral adipose depots after a long-term intake of grape seed proanthocyanidin extract in rats. *Br. J. Nutr.* 110: 1411–1420.

Sun Q, Prasad R, Rosenthal E and Katiyar SK (2011). Grape seed proanthocyanidins inhibit the invasive potential of head and neck cutaneous squamous cell carcinoma cells by targeting EGFR expression and epithelial-to-mesenchymal transition. *BMC Complement. Altern. Med.* 11: 134.

Sun T, Chen QY, Wu LJ, Yao XM, et al. (2012). Antitumor and antimetastatic activities of grape skin polyphenols in a murine model of breast cancer. *Food Chem. Toxicol.* 50: 3462–3467.

Tang K, Zhan JC, Yang HR and Huang WD (2010). Changes of resveratrol and antioxidant enzymes during UV-induced plant defense response in peanut seedlings. *J. Plant Physiol.* 167: 95–102.

Toaldo IM, Cruz FA, Alves TL, Gois JS, et al. (2015). Bioactive potential of *Vitis labrusca* L. grape juices from the Southern Region of Brazil: Phenolic and elemental composition and effect on lipid peroxidation in healthy subjects. *Food Chem.* 173: 527–535.

Ulusoy S, Ozkan G, Yucesan FB, Ersöz Ş, et al. (2012). Anti-apoptotic and anti-oxidant effects of grape seed proanthocyanidin extract in preventing cyclosporine A-induced nephropathy. *Nephrology.* 17: 372–379.

Zhang M, Zhou X and Zhou K (2013). Resveratrol inhibits human nasopharyngeal carcinoma cell growth via blocking pAkt/p70S6K signaling pathways. *Int. J. Mol. Med.* 31: 621–627.

Zhang XY, Li WG, Wu YJ, Bai DC, et al. (2005). Proanthocyanidin from grape seeds enhances doxorubicin-induced antitumor effect and reverses drug resistance in doxorubicin-resistant K562/DOX cells. *Can. J. Physiol. Pharmacol.* 83: 309–318.

Zhang ZZ, Li XX, Chu YN, Zhang MX, et al. (2012). Three types of ultraviolet irradiation differentially promote expression of shikimate pathway genes and production of anthocyanins in grape berries. *Plant Physiol. Biochem.* 57: 74–83.

Zheng M, Chen R, Zhong H, Lin Q, et al. (2012). Side-effects of resveratrol in HepG2 cells: Reduced *pten* and increased *bcl-xl* mRNA expression. *Mol. Med. Rep.* 6: 1367–1370.

Zhou K and Raffoul JJ (2012). Potential anticancer properties of grape antioxidants. *J. Oncol.* 2012: 1–8.