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Effects of the ingestion of different kinds of white grape juice (*Vitis labrusca*) during adolescence on body weight, biochemical parameters and oxidative stress in liver of adult Wistar rats

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ABSTRACT

The objective of this study was to evaluate the effects of the ingestion of different white grape juices: organic, conventional and conventional grape juice with 5% lemon juice during adolescence on biochemical serum profile and oxidative stress level in liver of adult Wistar rats. The phenolic and vitamin C composition of the juices were evaluated. During 32 days the rats were treated with the juices or oral water (gavage) for at a dose of 7 µL/g body weight. The animals were divided into 4 groups (n = 16/each). In the end, half of the animals received an intraperitoneal CCl_4 injection of 3.0 mL/kg; the other ones received mineral oil. After euthanasia, biochemical parameters were evaluated in serum and oxidative stress in the liver. It is possible to emphasize that the juices have different phenolic and vitamin C contents. The juice consumption didn't alter the weight body and biochemical parameters in adult life.

1. Introduction

Grape juice has an important role in the economy of Rio Grande do Sul. The consumption of grape juice has increased considerably throughout Brazil, the commercialization of this drink in the last 10 years has increased from 15.832.130 L in 2005 to 90.253.143 L in 2014, an increase of 570% (IBRAVIN, 2015)

The main varieties of grape juice in Brazilian production originate from grapes known as American and hybrid grapes from *Vitis labrusca* grapes such as Rose (goethe), Bordeaux and Concord (red), White Niagara and Moscato (white), varieties rich in phenolic compounds (Dani et al., 2007; IBRAVIN, 2015). There are two types of grapevine cultivation in Brazil. The organic, where no pesticides are used and the conventional style, with the presence of pesticides in the cultivation of the vine. According to a study by Toaldo et al. (2015), it was evidenced that organic grape juices have a higher concentration of phenolic compounds when compared to conventional juices (Toaldo et al., 2015).

The phenolic compounds have a chemical structure that lends them antioxidant properties (Tabeshpour, Mehri, Shaebani Behbahani, & Hosseinzadeh, 2018). Thus, these compounds have antioxidant, antimutagenic, anticancer, anti-teratogenic activity and assists in the immune response (Miglio et al., 2014).

Grapes stand out in the market because it is one of the fruits with the most phenolic compounds when compared to other fruits (Toaldo et al., 2015). Compared with wine, grape juice becomes the best alternative for the use of phenolic compounds, due to the absence of alcohol, allowing consumption by children, the elderly and pregnant women. A study carried out in animal model showed that grape juice performed its antioxidant and hepatoprotective activity against the oxidative damages resulting from the consumption of a high fat diet. Among the observed benefits, the reduction in fat accumulation within hepatocytes was identified (Buchner et al., 2014).

It is known that the role of food goes beyond the supply of nutrients and energy, they can promote beneficial physiological effects. The choice of polyphenol-rich food will bring benefits to human health and therefore must be consumed from adolescence to adulthood (Passos et al., 2017). Guaranteeing a diet rich in phenolic compounds from childhood can bring many benefits such as prevention of diseases such as cancer, obesity, cardiovascular diseases, among others (Di Daniele et al., 2017).

Phenolic compounds are not the only ones confer benefits to fruits

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and their derivatives, fruits also carry vitamins, notably vitamin C, which is characterized by being a natural antioxidant, obtained through diet (Liu, 2013). It is found primarily in citrus fruits such as tangerine, orange and lemon and acts as an antioxidant bringing great benefits to human health. Vitamin C, or ascorbic acid, acts as antioxidants on reactive oxygen species (ROS) and reactive nitrogen species (RNS) in biological environments. Acting directly on cell membranes preventing lipid peroxidation and indirectly on vitamin E regeneration, bringing many benefits due to its antioxidant potential (Vasconcelos et al., 2007).

To date, there are few studies in the literature that demonstrate the benefits of white grape juice to health. The search for alternatives that can enrich this benefit is a current market trend, and in this context, we see the introduction of white grape juice with the addition of lemon, in an attempt to increase its benefits by enriching it with vitamin C. In this way, the present study aims to evaluate the effects of the ingestion of different kinds of white grape juice, organic, conventional and conventional with the addition of lemon (*Vitis labrusca*) during adolescence on biochemical parameters and oxidative stress in liver of adult *Wistar* rats on carbon tetrachloride-induced damage.

2. Materials and methods

2.1. Juices

All the white grape juice were offered by Vinícola Sinuelo, located in the municipality of São Marcos, RS, Brazil. The conventional grape juice with lemon had 5% lemon juice added. Periods of validity were observed, and the same brands were used for the entire study. The juice was manufactured in 2015.

2.2. Analysis of phenolic compounds

The juice samples were diluted 1:100 in water for Folin-Ciocalteau analysis and 1: 2 in 40% ethanol for total flavonoids. For HPLC analysis, the pure samples were filtered on 45 µm pore Nylon membranes. The total content of phenolic compounds of the grape juices were measured using the colorimetric modification of Folin-Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Two hundred microliters of grape juice was assayed with 1000 µL of Folin-Ciocalteu reagent and 800 µL of sodium carbonate (7.5%, w/v). After 30 min, the absorbance was measured at 765 nm, and the results were expressed as mg/L catechin equivalent.

The analyzes of phenolic compounds were performed on HP model 1100 HPLC equipment, Lichrospher RP18 column (5 µm) equipped with a 210 nm UV detector and quaternary pump system. The reverse phase analysis consisted of: solvent A – water with 1% phosphoric acid and solvent B – Acetonitrile. The pumping system of the mobile phase was gradient, with 90% of solvent A from 0 to 5 min, 60% of A from 5 to 40 min and 90% of A from 45 to 50 min. The standard flow was maintained at 0.5 mL/min according to the literature (Lemos Lima Morelli Engenheira de Alimentos Doutor Marcelo Alexandre Prado Orientador, n.d.). The samples were filtered on Nylon membranes 0.45 µm in pore diameter. The phenolic compounds were identified according to their elution order and by comparing their retention time with those of their pure standards. The quantification was performed by the external standardization method, by correlating the area (mAU*s) of the compound peak to the standard curve performed with each standard evaluated (gallic acid, catechin, chlorogenic acid, epicatechin, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin and vitexin).

2.3. Animals

In our study, we used 80 male Wistar rats at 21 days of age, weighing about 200 g, coming from the Centro Universitário Metodista – IPA. They were maintained at 22 ± 1 °C, on a 12-h light dark cycle. The animals received the commercial feed. It was composed of 20,5% protein – predominantly soy, 54% carbohydrate, 4% lipid, 4.5% fiber, 7% ash and 10% moisture.

2.4. Treatment

Wistar rats were treated daily (once daily) with conventional, conventional organic white grape juice with addition of 5% lemon juice or water fed orally (gavage) for 32 days at the dose of 7 µL/g body weight. The animals were initially distributed in four experimental groups (n = 16 each), being: control, organic grape juice, conventional grape juice and conventional grape juice with the addition of lemon juice. All animals were kept 7 days in the adaptation phase, being fed with water and feed. When they were 28 days old, the treatment was started until 60 days of age, the rats received the juices orally (gavage). Body weight gain were evaluated weekly. After the end of the treatment, prior to the injections, they were subdivided into another 8 groups (n = 8 each) and each group received single dose injections of tetrachloride or mineral oil according to the designated group, in a single dose of 3.0 mL/kg body weight intraperitoneally (Gabardo et al., 2015). After 4 h of the injection, the animals were immediately euthanized with halothane, associated with exsanguination. Blood was collected by cardiac puncture and afterwards centrifuged and stored. The liver was removed and homogenized in KCL solution, the samples (liver and blood) were collected and stored for further testing. We evaluated biochemical parameters and the oxidative stress levels in the plasma (TBARS, Carbonilas, SOD, CAT and Sulfhydriles). These procedures adopted for these animals complied with the "Principles of care of laboratory animals of NIH" and Law 11.794/2008, according to guidelines of CONCEA and CEUA. The approval number of the research project at CEUA was 003/2016.

2.5. Biochemical parameters

The assays were performed in plasma by automation (Bioclin-BS120). To evaluate the lipid profile, total cholesterol (CT) (mg/dL), triglycerides (TG) (mg/dL) and high-density lipoprotein (HDL) (mg/dL) were used. Hepatic function was assessed by aspartate aminotransferase (AST) and alanine aminotransferase (ALT) analysis. Urea (mg/dL) and creatinine (mg/dL) were used as parameters for the evaluation of renal function.

2.6. Evaluation of oxidative stress parameters

2.6.1. Thiobarbituric acid reactive substances (TBARS) measurement

TBARS was used to determine lipid peroxidation. Trichloroacetic acid (10% w/v) was added to the homogenate to precipitate proteins and to acidify samples (Wills, 1966). This mixture was then centrifuged (1000 *g*, 3 min). The protein-free sample was extracted, and thiobarbituric acid (0.67% w/v) was added to the reaction medium. Tubes were placed in a water bath (100 °C) for 30 min. Absorbance was read at 535 nm in a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments). Commercially available malondialdehyde was used as a standard. Results were expressed as mmol/mg of protein.

2.6.2. Carbonyl assay

Carbonyl assay was used to determine oxidative damage to proteins. Homogenates were incubated with 2,4dinitrophenylhydrazine (DNPH 10 mmol/L) in 2.5 mol/L HCl solution for 1 h at room temperature, in the dark. Samples were vortexed every 15 min (Levine et al., 1990). Then 20% TCA (w/v) solution was added in tube samples, left in ice for 10 min and centrifuged for 5 min at 1000g, to collect protein precipitates. Another wash was performed with 10% TCA. The pellet was washed 3 times with ethanol: ethyl acetate (1:1) (v/v). The final precipitate were dissolved in 6 mol/L guanidine hydrochloride solution, left for 10 min at 37 °C, and read at 360 nm (Reznick, Packer, 1994) (T80 UV/VIS Spectrometer, PG Instruments).

2.6.3. Sulfhydryl assay

This assay is based on the reduction of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) by thiols, generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm (Aksenov & Markesbery, 2001). Briefly, 0.1 mM DTNB was added to 120 µL of the samples. This was followed by a 30 min incubation at room temperature in a dark room. Absorption was measured at 412 nm (T80 UV/VIS Spectrometer, PG Instruments). The sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were reported as nmol/mg protein.

2.6.4. Determination of antioxidant enzyme activities

Superoxide dismutase (SOD) activity, expressed as USOD/mg protein, was based on the inhibition of the ratio of autocatalytic adrenochrome formation at 480 nm (T80 UV/VIS Spectrometer, PG Instruments) (Bannister & Calabrese, 1987). Catalase (CAT) activity was determined by following the decrease in 240 nm absorption of hydrogen peroxide (H2O2). (T80 UV/VIS Spectrometer, PG Instruments). It was expressed as UCAT/mg protein (Aebi, 1984).

2.6.5. Dosage of proteins

Protein concentrations were determined by the method of Lowry, Rosebrouh, Lewis-Farr, and Randall (1951), using bovine serum albumin as standard (Lowry et al., 1951).

2.7. Statistical analysis

The results were expressed as mean and standard error of the mean. The normality of the data was expressed by the Kolmogorov-Smirnov test. The analysis of the phenolic composition and vitamin C were performed by one-way ANOVA, followed by Tukey's post-test. For the other analyses the differences between the groups were examined by two-way ANOVA followed by Tukey's test post, considering a statistical difference when $p < 0.05$. All analyzes were performed using SigmaPlot 11.0 statistical software (Systat Software, San Jose, CA, USA).

3. Results and discussion

The total phenolic compounds of the white grape juice *Vitis Labrusca L.* were quantified by Folin-Ciocalteau and identified individually by high efficiency chromatography (HPLC) (Table 1). We observed that the concentration of total phenolic compounds (TPC) and catechin was higher in the organic juice when compared to the conventional variety and the conventional juice + lemon ($p < 0.05$). In relation to resveratrol, we observed that conventional juice presented higher concentration when compared to the organic and the conventional + lemon juice. When the concentration of vitamin C was higher in the organic juice (Table 1).

The phenolic compounds present in the grape can act as natural antioxidants (Dani et al., 2007). In our study we observed that the concentration of total phenolic compounds and of catechin in organic white grape juice was higher compared to the other grape juice varieties. Studies have identified that organic grape juice made from white and red grapes of the *Vitis labrusca* variety showed a significantly higher concentration of phenolic compounds when compared to grape juice obtained through conventional cultivation (Dani et al., 2007; Rodrigues et al., 2012). In the study by Toaldo et al. (2015), when analyzing the phenolic composition of organic and conventional red grape juices and conventional white juice, the concentrations of anthocyanins, flavonoids and phenolic acids were significantly higher in red organic juice (Toaldo et al., 2015).

In our study, resveratrol was present in higher concentration in conventional juice followed by organic and conventional + lemon. A similar result was also observed in the study by Freitas, Detoni, Clemente, & Oliveira (2010), where the concentration of resveratrol in red grape juice was higher in conventional juice (32,5 mg L^1) than in organic juice (25,9 mg L^1). In contrast, another study observed a higher concentration of resveratrol in organic red juice when compared to conventional red juice and conventional white juice (Toaldo et al., 2015). Resveratrol is a non-flavonoid with great antioxidant potential, found in large concentrations on the peel of the grapes, mainly of the bordeaux variety of *Vitis labrusca L* and in smaller concentration in the white grapes (Rauf et al., 2017).

When analyzing concentration of vitamin C (ascorbic acid), the highest concentration was organic grape juice and finally the conventional juices. Our results are in accordance with Rodrigues et al. (2012), where organic grape juice presented higher concentration of ascorbic acid compared to conventional grape juice. Vitamin C promotes regeneration of the membrane-bound alpha-tocopherol radical and removes the radical for the aqueous phase, protecting the tissues from lipid peroxidation both *in vivo* and *in vitro* (Oudemans-van Straaten, Spoelstra-de Man, & de Waard, 2014; Pari & Suresh, 2008). In a study by González-Molina and colleagues (2012), aiming to analyze new drinks rich in polyphenols, such as grape juice or elderberry concentrate combined with 5% lemon juice, the phytochemical composition and antioxidant capacity of these juices was analyzed. It was verified that grape juice and elderberry concentrate had higher antioxidant activity with addition of lemon juice, as well as a higher concentration of anthocyanins (González-Molina, Gironés-Vilaplana, Mena, Moreno, & García-Viguera, 2012).

In the five weeks of treatment in our study, all animals had weight gain, but there was no statistical difference in weight gain between the groups (Fig. 1). Similarly, in a study with rats submitted to organic and conventional red grape juice consumption during twelve weeks of treatment, no significant change in rodent body weight between the groups studied was identified (Hollis, Houchins, Blumberg, & Mattes,

Table 1
Content of total phenolic compounds, isolated phenolic compounds and vitamin C content in white grape juice.

Phenolic compound (mg/L)	Organic	Conventional	Conventional + Lemon
Total Phenolic Compound	475.49 ± 2.450*a	460.88 ± 6.50	436.27 ± 6.48
Catechin	0.610 (0.610–0.617)*b	0.310 (0.310–0.318)	0.310 (0.310–0.318)
Epicatechin	0.610 (0.610–0.625)	0.630 (0.623–0.630)	0.620 (0.613–0.620)
Resveratrol	0.560 ± 0.025	0.817 ± 0.012*	0.673 ± 0.029
Vitexin	1.20 ± 0,039	2.66 ± 0.030	2.16 ± 0.026
Hesperidin	NE	37.47 (36.87–37.88)	35.26 (35.18–35.36)
Flavonoids Total	6.26 ± 0.14	8.08 ± 0.18	9.17 ± 0.15
Chlorogenic Acid	NE	3.89 ± 0.024	3.75 ± 0.14
Ferulic Acid	NE	13.66 ± 0.03	11.6 ± 0.05
Vitamin C (mg/dL)	19.23 ± 0.32*	4.80 ± 0.17	5.70 ± 0.17

(a) Results expressed as mean ± standard error of the mean. (b) Results expressed in median (25–75). NE: Not Found. *Statistical difference with the other juices according to ANOVA, followed by Tukey test post, $p < 0.05$.

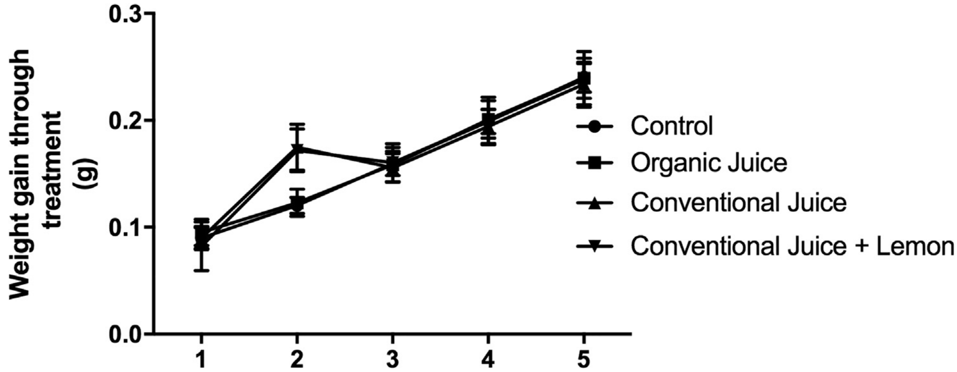

Fig. 1. Weight gain over treatment. Results expressed as measure/g weight of male Wistar rats treated with different white grape juices.

2009). However, in the study by Buchner et al. (2014) the body composition of rats was evaluated after 12 weeks of treatment with a hyper-lipidic diet, conventional red grape juice and/or organic grape juice, and it was observed that the animals treated with a hyper-lipidic diet associated with conventional or organic grape juice presented lower body weight, with the most significant reduction being associated to organic juice group (Buchner et al., 2014). According to other studies in the literature, maintaining a daily intake of grape juice, a polyphenol-rich drink, associated with a balanced diet does not cause significant increases in body weight (Krikorian, Nash, Shidler, Shukitt-Hale, & Joseph, 2010). This fact is very important because some studies are showing the soft beverages consumption during the childhood have contributed to the obesity in the adult life, but the 100% juice, without sugar added could protect this scenario (Eshak et al., 2013).

We observed alterations in the lipid profile regarding the animals that consumed the juices (Table 2). Regarding total cholesterol (CT), levels presented an increase in the oil group (60.53 ± 1.50 mg/dL) in relation to the CCl_4 group (54.26 ± 1.57 mg/dL). In relation to HDL, it was observed that levels decreased in the groups that received conventional juice and conventional + lemon juice ($CCl_{4)}$ when compared to the respective oil group. There were no statistical differences between groups as far as triglyceride levels were concerned. When we analyzed hepatic function through AST and ALT, we observed that animals receiving the dose of CCl_4 (220 ± 9.95 U/L) had higher AST levels than the oil group (113.646 ± 8.828 U/L); the same happened with ALT, where we observed that animals that received CCl_4 (73.28 ± 2.65 U/L) presented higher levels than the oil groups (56.93 ± 2.62 U/L). For both enzymes we observed that the juices did not protect the damage induced by CCl_4 (Table 2). In relation to creatinine, it was observed that the levels decreased in the CCl_4 group (0.165 ± 0.012 mg/dL) compared to the oil group (0.272 ± 0.012 mg/dL), the juices failed to protect against the damage caused by CCl_4. No significant differences were observed between the groups in relation to urea on these same experimental groups.

When evaluating the biochemical profile, it is possible to observe that the CT, levels remained the same, except for animals that consumed lemon juice associated with CCl_4. In a study with leaf extract of the Bordo grape variety, it was also observed that the daily intake of the extract via gavage for thirty days did not alter the levels of CT in diabetic rats (Lacerda et al., 2014). On the other hand, HDL levels decreased in the groups that received conventional and conventional + lemon juice in our study associated with CCl_4. In contrast to our study, certain studies (however analyzing the consumption of red grape juice) demonstrated significant increases in HDL (Ahmadi & Sk, 2014; Aminian, Aminian, & Hoseinali, 2006). There was no statistical difference the triglyceride (TG) levels in the groups. This is in agreement with a study by Aminian and collaborators (2006), where the consumption of red grape juice in hypercholesterolemic rabbits did not alter TG and HDL levels. The AST and ALT markers were used to evaluate hepatic function in rats (Aminian et al., 2006). The hepatic changes caused by CCl_4 were evidenced by the increase in activity of these enzymes. These results are in line with those of Hermenean and collaborators (2017) where twenty-four hours after administration of CCl_4 an increase in the activity levels of AST and ALT was verified in a study with chrysin, a natural flavonoid extracted from the honey of plants (Hermenean et al., 2017).

Although in this study, the juice did not protect against liver damage caused by CCl4, there are several other studies which demonstrated the hepatoprotection offered by grapes and its by products, including grape juice, both *in vivo* and *in vitro* (Buchner et al., 2014; Dani, Pasquali, et al., 2008; Lacerda et al., 2014; Oliboni, Dani, Funchal, Henriques, & Salvador, 2011; Rodrigues et al., 2013). The process of

Table 2
Determination of biochemical parameters, hepatic and renal markers in plasma of adult Wistar rats treated with conventional, conventional organic grape juice with addition of 5% lemon.

Group		Parameters						
Beverage	Ag.	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	AST (U/L)	ALT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	Oil	61.37 ± 3.32	88.90 ± 10.12	36.41 ± 1.51	127.37 ± 19.46	64.62 ± 5.78	58.26 ± 4.35	0.276 ± 0,027
Organic	Oil	55.00 ± 3.32	80.31 ± 10.12	34.23 ± 1.51	126.12 ± 19.46	58.62 ± 5.78	56.16 ± 4.35	0.224 ± 0.026
Conventional	Oil	59.37 ± 3.32	82.15 ± 10.12	37.08 ± 1.51	111.00 ± 19.46	59.37 ± 5.77	60.46 ± 4.35	0.261 ± 0.027
Conv + Lemon	Oil	61.62 ± 3.32	89.11 ± 10.12	38.46 ± 1.51	109.87 ± 19.46	54.62 ± 5.78	59.35 ± 4.35	0.295 ± 0,027
Lemon	Oil	65.28 ± 3.55	96.31 ± 10.82	40.13 ± 1.61	93.86 ± 20.81	47.43 ± 6.18	58,30 ± 4.65	0,304 ± 0,0288
Control	CCl_4	51.71 ± 3.55	83.84 ± 10.82	30.44 ± 1.61	198.57 ± 20.81*	72.57 ± 6.18*	51.57 ± 4,65	0.123 ± 0.028*
Organic	CCl_4	49.28 ± 3.55	91.03 ± 10.12	30.04 ± 1.51	225.87 ± 19.46*	77.62 ± 5.78*	66.41 ± 4.35	0.140 ± 0.027*
Conventional	CCl_4	51.75 ± 3.32	78.11 ± 10.12	31.83 ± 1.51*	225.87 ± 19.46*	71.75 ± 5.78*	54.30 ± 4.35	0.169 ± 0.027*
Conv + Lemon	CCl_4	52.14 ± 3.55	83.27 ± 10.12	31.53 ± 1.51*	225.87 ± 19.46*	83.50 ± 5.78*	63.44 ± 4.35	0.184 ± 0.027*

Values expressed as mean ± standard error of the mean.

* Statistical difference when comparing with the same group with other agent according to two-way ANOVA, followed by Tukey test post, $p < 0.05$. HDL: high-density lipoprotein, AST: aspartate aminotransferase and ALT: alanine aminotransferase.

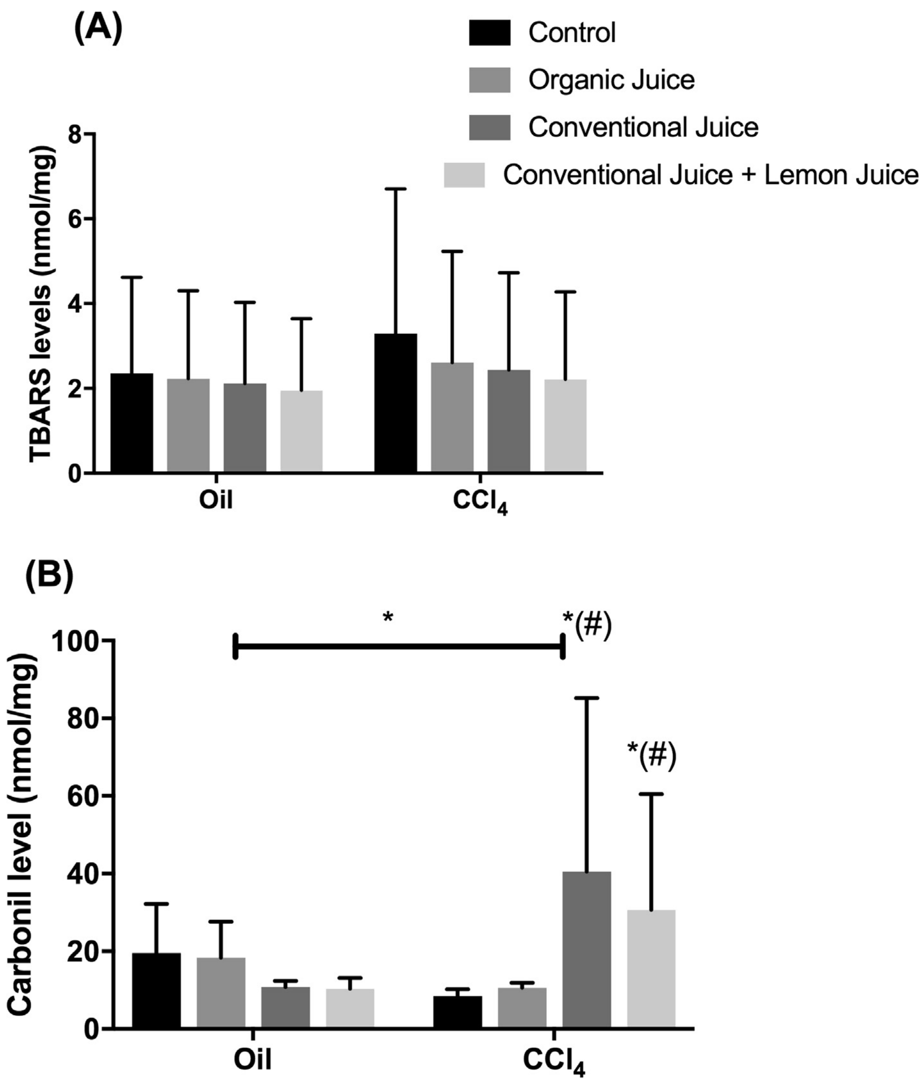

Fig. 2. Levels of TBARS (nmol/mg) and Carbonyl (nmol/mg), expressed as mean ± standard error of the liver mean of male Wistar rats treated treated with different white grape juices. *Difference of CCl_4 group with their respective in the oil group. **Difference oil group with your control. #Difference of group CCl_4 with your control. Statistical difference according to two-way ANOVA, followed by Tukey test, $p < 0.05$.

oxidative stress is characterized when there is an imbalance between excess free radical production versus the antioxidant defense mechanisms (Tromm et al., 2012). According to Tromp and collaborators (2012), oxidative stress alters the physiological functioning of several organs, such as the liver and heart, causing alterations in liver and heart cells, causing diseases such as hepatitis C, atherosclerosis and hepatic steatosis.

Regarding the levels of lipid peroxidation (TBARS) in the liver, no significant differences were observed between the treated groups (Fig. 2). In relation to protein oxidation (Carbonil), it was observed that the CCl_4 group (34.67 ± 4.26 nmol/mg) had higher values than the oil group (18.20 ± 4.45 nmol/mg). In the CCl_4 group, conventional and conventional + lemon juice presented high carbonyl values in relation to the control. These same juices showed high carbonyl levels when compared to their respective ones in the oil group (Fig. 2). There were no statistically significant differences between the groups treated with white grape juice with or without CCl_4.

In our study, a few markers of oxidative stress were measured using Carbonil and TBARS techniques in the liver of adult *Wistar* rats. We observed that in the liver the levels of protein oxidation in the group that received the damage inducer did not present significant differences in relation to the oil group. In the CCl_4 group, the conventional and conventional + lemon juices failed to prevent the damage caused by CCl_4, but the organic juice group we didn't see this increase. In contrast, Buchner et al. (2014) observed a protection, however with organic and conventional red juices, in a model of obesity (Buchner et al., 2014). When evaluating the levels of lipid peroxidation (TBARS) no statistical differences were observed in the liver of the evaluated groups. However, in a study of the group, it was found that organic and conventional grape juice was able to reduce the levels of TBARS, providing protection to the liver tissue of Wistar rats (Lacerda et al., 2014).

When evaluating the enzymatic activity of SOD in the liver we observed no statistical difference between the oil and CCl_4 groups. However, within the CCl_4 group, all juices increased the activity of the SOD enzyme when compared to the control (Fig. 3). In addition to evaluating SOD activity, we evaluated the antioxidant enzymatic defenses through CAT. However, no statistical differences were observed in the liver of the evaluated groups ($p > 0.05$) (Fig. 3).

When there is an overproduction of free radicals in the body, the antioxidant system is triggered. Our body has an efficient antioxidant system that can control and reestablish balance (Vasconcelos et al., 2007). The accumulation of free radicals in our body can be avoided through the enzymatic defenses of SOD and CAT (Pari & Suresh, 2008). In our study in the CCl_4 group, there was an increase in the SOD enzyme activity with all juices when compared to the control. This agrees with the literature when they observed the protective effects of hesperidin, a

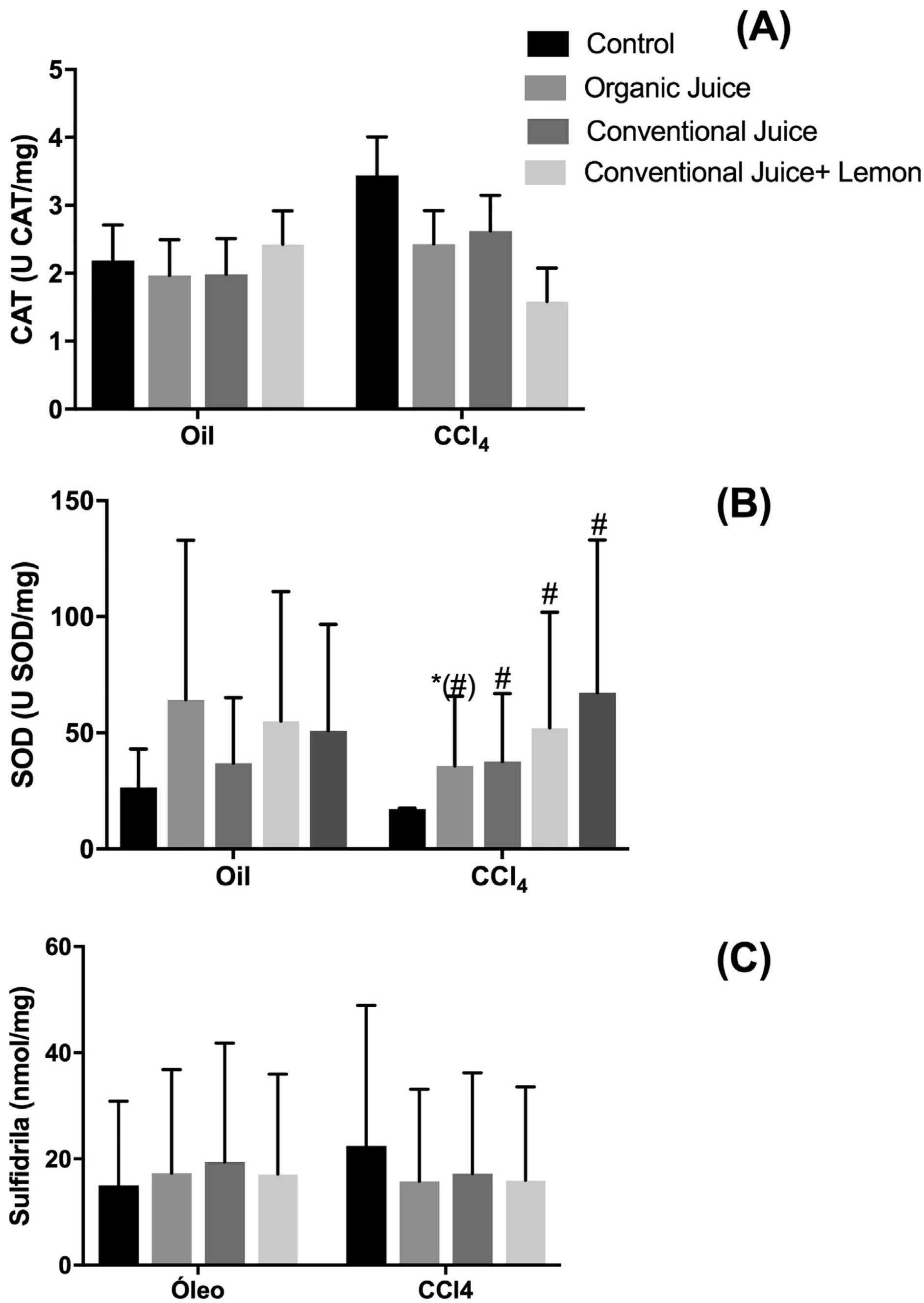

Fig. 3. Levels of SOD (U SOD/mg), CAT (U CAT/mg) and Sulfhydryl (nmol/mg), expressed as mean ± standard error of the liver mean of male Wistar rats treated with treated with different white grape juices.*Difference of group CCl_4 with its respective in the oil group. **Difference oil group with your control. #Difference of group CCl_4 with your control. Statistical difference according to two-way ANOVA, followed by Tukey test, $p < 0.05$.

flavonoid with antioxidant action over oxidative stress, dyslipidemia and histological alterations due to hepatic and renal toxicity induced by iron in rats (Pari & Suresh, 2008). In this study the association of iron-associated hesperidin increased the levels of enzymatic antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) in liver and kidney. In another study, consumption of organic and conventional red grape juice inhibited induced reduction by PTZ in enzymatic antioxidant defenses SOD and CAT in liver and serum (Rodrigues et al., 2012). In the CAT activity, no statistical differences were observed in the liver of the evaluated groups. However, in a study with rats treated with organic and conventional red juice induced to CCl_4 damage, there was an increase in CAT activity in liver and serum of *Wistar* rats (Dani, Oliboni, et al., 2008). In the present study, sulfhydryl levels had no significant change between groups. This fact was also evidenced in a study where there was no statistical difference in the levels of sulfhydryl in the serum and liver of Wistar rats (Farias Wohlenberg et al., 2015).

4. Conclusion

Our results reinforce the evidence that white grape juices present an important concentration of phenolic compounds, albeit in a different concentration depending on juice type. The white grape juice consumption, with or without lemon juice, during the adolescence didn't alter the weight body in the rat's adult life. The organic grape juice consumption didn't alter the carbonyl level different from the other grape juices, this fact could be explain by the phenolic content level. We believe grapes receive this merit for presenting a high concentration of phenolic compounds that bring many health benefits, from adolescence to adulthood in experimental model. This study provides results in a Wistar rats model, to obtain a complete approach on the hepatic benefits of grape juice, further studies are needed.

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Conflict of interest

The authors declare no conflicts of interest.

References

Aebi, H. (1984). [13] Catalase in vitro. *Methods in Enzymology, 105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Ahmadi, L., & Sk, R. (2014). Pharmacological and Phytochemical Properties of Unripe Grape Juice (Verjuice): A Review. 1(2).

Aksenov, M. Y., & Markesbery, W. R. (2001). Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. *Neuroscience Letters, 302*(2–3), 141–145.

Aminian, A., Aminian, B., & Hoseinali, A. A. N. F. (2006). Acta medica Iranica. In Acta Medica Iranica. Retrieved from http://acta.tums.ac.ir/index.php/acta/article/view/3189.

Bannister, J. V., & Calabrese, L. (1987). Assays for superoxide dismutase. *Methods of Biochemical Analysis, 32*, 279–312.

Buchner, I., Medeiros, N., Lacerda, D. D. S., Normann, C. A. B. M., Gemelli, T., Rigon, P., ... Funchal, C. (2014). Hepatoprotective and antioxidant potential of organic and conventional grape juices in rats fed a high-fat diet. *Antioxidants (Basel, Switzerland), 3*(2), 323–338. https://doi.org/10.3390/antiox3020323.

Dani, C., Oliboni, L. S., Pasquali, M. A. B., Oliveira, M. R., Umezu, F. M., Salvador, M., ... Henriques, J. A. P. (2008a). Intake of purple grape juice as a hepatoprotective agent in Wistar rats. *Journal of Medicinal Food, 11*(1), 127–132. https://doi.org/10.1089/jmf.2007.558.

Dani, C., Oliboni, L. S., Vanderlinde, R., Bonatto, D., Salvador, M., & Henriques, J. A. P. (2007). Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. *Food and Chemical Toxicology, 45*(12), 2574–2580. https://doi.org/10.1016/j.fct.2007.06.022.

Dani, C., Pasquali, M. A. B., Oliveira, M. R., Umezu, F. M., Salvador, M., Henriques, J. A. P., & Moreira, J. C. F. (2008b). Protective effects of purple grape juice on carbon tetrachloride-induced oxidative stress in brains of adult Wistar rats. *Journal of Medicinal Food, 11*(1), 55–61. https://doi.org/10.1089/jmf.2007.505.

Di Daniele, N., Noce, A., Vidiri, M. F., Moriconi, E., Marrone, G., Annicchiarico-Petruzzelli, M., ... De Lorenzo, A. (2017). Impact of Mediterranean diet on metabolic syndrome, cancer and longevity. *Oncotarget, 8*(5), 8947–8979. https://doi.org/10.18632/oncotarget.13553.

Eshak, E. S., Iso, H., Mizoue, T., Inoue, M., Noda, M., & Tsugane, S. (2013). Soft drink, 100% fruit juice, and vegetable juice intakes and risk of diabetes mellitus. *Clinical Nutrition (Edinburgh, Scotland), 32*(2), 300–308. https://doi.org/10.1016/j.clnu.2012.08.003.

Farias Wohlenberg, M., Gonçalves, L. K., Schaffer, T. K., Hilger, D. K., Dario, R., Neto, B., ... Dani, C. (2015). Effect of grape juice on some biochemical and oxidative stress parameters in serum and liver enzymes of pregnant and lactating rats. *Issues in Biological Sciences and Pharmaceutical Research, 3*(4), 37–46. https://doi.org/10.15739/ibspr.011.

Freitas, A. A. de, Detoni, A. M., Clemente, E., & Oliveira, C. C. de (2010). Determinação de resveratrol e características químicas em sucos de uvas produzidas em sistemas orgânico e convencional. *Revista Ceres, 57*(1), 1–5. https://doi.org/10.1590/S0034-737X2010000100001.

Gabardo, T., Peripolli, C. M., de Andrade, R. B., Gemelli, T., Lima, J. D. O., Oliveira, A. S., ... Funchal, C. (2015). Assessment of changes in energy metabolism parameters provoked by carbon tetrachloride in Wistar rats and the protective effect of white grape juice. *Toxicology Reports, 2*. https://doi.org/10.1016/j.toxrep.2015.03.011.

González-Molina, E., Gironés-Vilaplana, A., Mena, P., Moreno, D. A., & García-Viguera, C. (2012). New beverages of lemon juice with elderberry and grape concentrates as a source of bioactive compounds. *Journal of Food Science, 77*(6), C727–C733. https://doi.org/10.1111/j.1750-3841.2012.02715.x.

Hermenean, A., Mariasiu, T., Navarro-González, I., Vegara-Meseguer, J., Miuțescu, E., Chakraborty, S., & Pérez-Sánchez, H. (2017). Hepatoprotective activity of chrysin is mediated through TNF-α in chemically-induced acute liver damage: An in vivo study and molecular modeling. *Experimental and Therapeutic Medicine, 13*(5), 1671–1680. https://doi.org/10.3892/etm.2017.4181.

Hollis, J. H., Houchins, J. A., Blumberg, J. B., & Mattes, R. D. (2009). Effects of concord grape juice on appetite, diet, body weight, lipid profile, and antioxidant status of adults. Retrieved from *Journal of the American College of Nutrition, 28*(5), 574–582. http://www.ncbi.nlm.nih.gov/pubmed/20439553.

IBRAVIN (2015). No Title.

Krikorian, R., Nash, T. A., Shidler, M. D., Shukitt-Hale, B., & Joseph, J. A. (2010). Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *The British Journal of Nutrition, 103*(5), 730–734. https://doi.org/10.1017/S0007114509992364.

Lacerda, D. D. S., Santos, C. F., Oliveira, A. S., Zimmermann, R., Schneider, R., Agostini, F., ... Gomez, R. (2014). Antioxidant and hepatoprotective effects of an organic grapevine leaf (*Vitis labrusca* L.) extract in diabetic rats. *RSC Advances, 4*(95), https://doi.org/10.1039/c4ra08396b.

Lemos Lima Morelli Engenheira de Alimentos Doutor Marcelo Alexandre Prado Orientador, L. (n.d.). AVALIAÇÃO DE COMPOSTOS FENÓLICOS EM GELEIA DE UVA PRODUZIDA COM A VARIEDADE IAC-138-22 (MÁXIMO). Retrieved from http://repositorio.unicamp.br/jspui/bitstream/REPOSIP/254817/1/Morelli_LuciulaLemosLima_M.pdf.

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., ... Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology, 186*, 464–478.

Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition (Bethesda Md.), 4*(3), 384S–392S. https://doi.org/10.3945/an.112.003517.

Lowry, O. H., Rosebrouh, N. J., Lewis-Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry, 193*, 265–275. https://doi.org/10.1016/0304-3894(92)87011-4.

Miglio, C., Peluso, I., Raguzzini, A., Villaño, D. V., Cesqui, E., Catasta, G., ... Serafini, M. (2014). Fruit juice drinks prevent endogenous antioxidant response to high-fat meal ingestion. *British Journal of Nutrition, 111*(02), 294–300. https://doi.org/10.1017/S0007114513002407.

Oliboni, L. S., Dani, C., Funchal, C., Henriques, J. A., & Salvador, M. (2011). Hepatoprotective, cardioprotective, and renal-protective effects of organic and conventional grapevine leaf extracts (*Vitis labrusca* var. Bordo) on Wistar rat tissues. *Anais Da Academia Brasileira de Ciencias, 83*(4).

Oudemans-van Straaten, H. M., Spoelstra-de Man, A. M., & de Waard, M. C. (2014). Vitamin C revisited. *Critical Care (London, England), 18*(4), 460. https://doi.org/10.1186/s13054-014-0460-x.

Pari, L., & Suresh, A. (2008). Effect of grape (Vitis vinifera L.) leaf extract on alcohol induced oxidative stress in rats. *Food and Chemical Toxicology, 46*(5), 1627–1634. https://doi.org/10.1016/J.FCT.2008.01.003.

Passos, J. A., Freitas, M. do C. S. de, Santos, L. A. da S., Soares, M. D., Passos, J. A., Freitas, M. do C. S. de, & Soares, M. D. (2017). Meanings attributed to healthy eating by consumers of a street market. *Revista de Nutrição, 30*(2), 261–270. https://doi.org/10.1590/1678-98652017000200010.

Rauf, A., Imran, M., Suleria, H. A. R., Ahmad, B., Peters, D. G., & Mubarak, M. S. (2017). A comprehensive review of the health perspectives of resveratrol. *Food & Function, 8*(12), 4284–4305. https://doi.org/10.1039/c7fo01300k.

Rodrigues, A. D., Scheffel, T. B., Scola, G., dos Santos, M. T., Fank, B., Dani, C., ... Salvador, M. (2013). Purple grape juices prevent pentylenetetrazol-induced oxidative damage in the liver and serum of Wistar rats. *Nutrition Research, 33*(2), https://doi.org/10.1016/j.nutres.2012.12.002.

Rodrigues, A. D., Scheffel, T. B., Scola, G., Santos, M. T. D., Fank, B., De Freitas, S. C. V., ... Salvador, M. (2012). Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole. *Neurochemistry International, 60*(8), https://doi.org/10.1016/j.neuint.2012.01.009.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology, 299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

Tabeshpour, J., Mehri, S., Shaebani Behbahani, F., & Hosseinzadeh, H. (2018). Protective effects of *Vitis vinifera* (grapes) and one of its biologically active constituents, resveratrol, against natural and chemical toxicities: A comprehensive review. *Phytotherapy Research, 32*(11), 2164–2190. https://doi.org/10.1002/ptr.6168.

Toaldo, I. M., Cruz, F. A., Alves, T. D. L., De Gois, J. S., Borges, D. L. G., Cunha, H. P., ... Bordignon-Luiz, M. T. (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 Chemistry, 173*, 527–535. https://doi.org/10.1016/j.foodchem.2014.09.171.

Tromm, C. B., Da Rosa, G. L., Bom, K., Mariano, I., Pozzi, B., Tuon, T., ... Pinho, R. A. (2012). Efeito de diferentes frequências semanais de treinamento sobre parâmetros de estresse oxidativo. *Revista Brasileira de Cineantropometria e Desempenho Humano, 14*(1), 52–60. https://doi.org/10.5007/1980-0037.2012v14n1p52.

Vasconcelos, S. M. L., Goulart, M. O. F., Moura, J. B. de F., Manfredini, V., Benfato, M. da S., & Kubota, L. T. (2007). Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: Principais métodos analíticos para sua determinação. *Química Nova, 30*(5), 1323–1338. https://doi.org/10.1590/S0100-40422007000500046.

Wills, E. D. (1966). Mechanisms of lipid peroxide formation in animal tissues. Retrieved from *Biochemical Journal, 99*.