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Effects of grape juice, red wine and resveratrol on liver parameters of rat submitted high-fat diet

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Abstract: This work evaluated the effect of grape juice, red wine and resveratrol in liver parameters of rats submitted to high-fat diet. Experimental model was conducted with groups of adult females *Rattus norvegicus*: control (CG); high-fat (HG); grape juice (JG); red wine (RW) and resveratrol solution (RG). The high-fat diet significantly altered hepatocytes and Kupffer cells in all treated groups. HG group presented severe steatosis followed hepatocyte ballooning and tissue damages. JG group minimized hepatic histological lesion caused by high-fat diet and WG group also induced steatosis and inflammation in hepatocytes, similar to HG. Still, resveratrol protected the tissue against fatty liver disease by reducing fat infiltration and inflammation, indicating possible therapeutic effects on the liver. Cell cycle analysis showed that HG promoted damage to the tissue, reducing the viable cell content and increasing apoptosis, even when associated with wine consumption or isolated resveratrol. However, JG protected the liver against cell damage generated by the diet. Consumption of grape juice, even associated with a high-fat diet, represents a promising protection of the liver against cellular damage, but red wine further affects the tissue, and resveratrol alone was able to reduce damage but did not minimize cellular damage to the liver.

Key words: Grape juice, high-fat diet, liver, resveratrol, wine.

INTRODUCTION

Diets play major role in both health care and disease progression (Pereira et al. 2014, Milić et al. 2014). The Consumption of a high-fat diet is associated with increased body weight, metabolic changes, such as inflammation and oxidative stress, cell damage as well as chronic non-transmissible diseases involving various tissues such as the liver. (Milić et al. 2014, Oarada et al. 2012, Bedê et al. 2015, Lozano et al. 2016).

Excessive intake of lipids induces increased flow and oxidation of fatty acids in the liver with increased fat deposition in hepatocytes and promoted inflammation and lipotoxicity contributing to cell malfunction, cell death and cell lesions (Cohen et al. 2011). Associated with a high-fat diet, alcohol consumption is also reported as an etiological factor for hepatic steatosis, as well as other drugs or toxins (Noureddin & Rinella 2015). Excessive fat deposition in the liver causes mitochondrial dysfunction and fatty acids oxidation produces high production of reactive oxygen species (ROS) as side product of that causes damage to cellular membranes, proteins, lipids and deoxyribonucleic acid (DNA) (Noureddin & Rinella 2015, Reynés 2015).

Chronic hepatic lipid accumulation results in non-alcoholic fatty liver disease (NAFLD) that may progress to non-alcoholic steatohepatitis (NASH), a condition characterized by chronic inflammation and fibrosis, associated with overweight. Hepatocytes respond to lesions by various mechanisms, such as apoptosis, non-apoptotic death and cellular autophagy (Widiker et al. 2010, Leung et al. 2016). However, external environmental factors have been studied, such as nutrients that damage or stimulate hepatic cells for regeneration. (Dudley et al. 2011, Kim et al. 2011). The high-fat diet leads to epigenetic changes in the hepatic tissue cell cycle (Czaja et al. 2013). Excess dietary fat leads to changes in chromatin and intracellular protein activation that influence the progression of different cycle phases. This can have a major impact on phenotypic results making cells vulnerable to apoptosis and favoring the inflammatory response in tissues (Mizushima et al. 2008, Wellenk & Thompson 2010). Oxidative stress triggered by ROS overproduction also activates the lysosomal cell death pathway and results in cytotoxicity, leading to hepatic inflammation (Noureddin & Rinella 2015, Bantel 2012).

Food consumption give several bioactive compounds that have health benefits (Laliena et al. 2012, Yuzefovych et al. 2013, Charradi et al. 2013, Rahal et al. 2014, Ghanim et al. 2011). Grapes are rich in polyphenols, whose consumption presents health benefits, which makes the fruit known for its functional properties. Studies in the liver tissue show that grape compounds provide tissue with pro-ion in both disease prevention and disease development. (Poljsak 2011, Cunha et al. 2016).

In this case, beverages derived from grapes, such as red wine and whole red grape juice, present a complex range of phenolic compounds, such as anthocyanins, resveratrol and quercetin, known not only for their strong antioxidant effect, but also for the prevention of oxidative reactions and formation of free radicals, and for their anti-proliferative and anti-inflammatory effects (Rindler et al. 2013, Giovinazzo & Grieco 2015, Singh et al. 2016).

Experimental models of diet-induced hepatotoxicity have contributed to elucidate the pathophysiology of various hepatic diseases and also aim to identify and evaluate possible hepatoprotective dietary agents (Milić et al. 2014, Oarada et al. 2012, Bedê et al. 2015, Lozano et al. 2016). It is therefore important to study the possible effect of consuming polyphenol-rich beverages under the liver that are attacked by a high-fat diet, evaluating their potential in liver cell regeneration.

Therefore, the aim of this study was to evaluate the effect of whole grape juice, red wine and resveratrol solution isolated in the hepatic tissue of rats submitted to the high fat diet, in relation to histological, cell cycle and apoptosis parameters.

MATERIALS AND METHODS

Experimental design and sampling

The study was conducted in the Laboratory of Experimental Nutrition at Department of Nutrition and Dietetics of Federal Fluminense University (LabNE-UFF). All animal procedures were approved by the Animal Ethics Committee from Federal Fluminense University (protocol under number 00216/10).

A total of 50 female Wistar rats, all adults (90 days), weighing 200±20g obtained at the LabNE-UFF were kept in cages in a controlled environment (24°±2°C, with a 12 h daylight cycle). The experiment lasted for 8 weeks.

Animals were randomly divided into five groups (n=10/group): 1) Control Group (CG): received standard diet (4% fat) based on the AIN-93M (Reeves et al 1993); 2) High- fat group

(HG): received high-fat diet (20% fat); 3) Grape juice group (JG): received high fat diet (20% fat) and whole grape juice (15 mL/day); 4) Red wine group (RW): received high fat diet (20% fat) and red wine (10 mL/day); 5) Resveratrol solution group (RG): received high fat diet (20% fat) and resveratrol solution (15 mL/day – 40 mg/L). All animals were given access to feed and water *ad libitum*. Juice, wine and resveratrol solution were offered in individual bottles. This dietary protocol is illustrated in Figure 1 and the Table I shows the composition of the diets of the different groups.

Red wine (*Cabernet Sauvignon*) and grape juice were obtained from local market and were kept refrigerated (10 °C) during the study. Considering the concentration of total polyphenols of the red wine (1014-3718 mg gallic acid equivalent (GAE)/L, mean 2366 mg GAE/L) and whole grape juice (1617–2213, mean 1915 mg GAE/L), the volume offered to the animals of grape juice (15mL/day) and wine (10mL/day) was determined to resemble the amount of polyphenols in the two drinks (Oliveira et al. 2009, Roesler et al. 2007, Sautter et al. 2005).

Resveratrol solution was prepared daily in the laboratory by dissolving the content in distilled water and adding 5% refined sugar. Final concentration (40 mg/L) of resveratrol solution was based in previous study, making the proportion of the dose to the weight of the animal (Timmers et al. 2011).

Body weight and chow intake of animals were recorded weekly using BioPrecisa® (B15-0.5, Labmais Ltda, PR, Brazil) precision scale. Beverage consumption (water, whole red grape
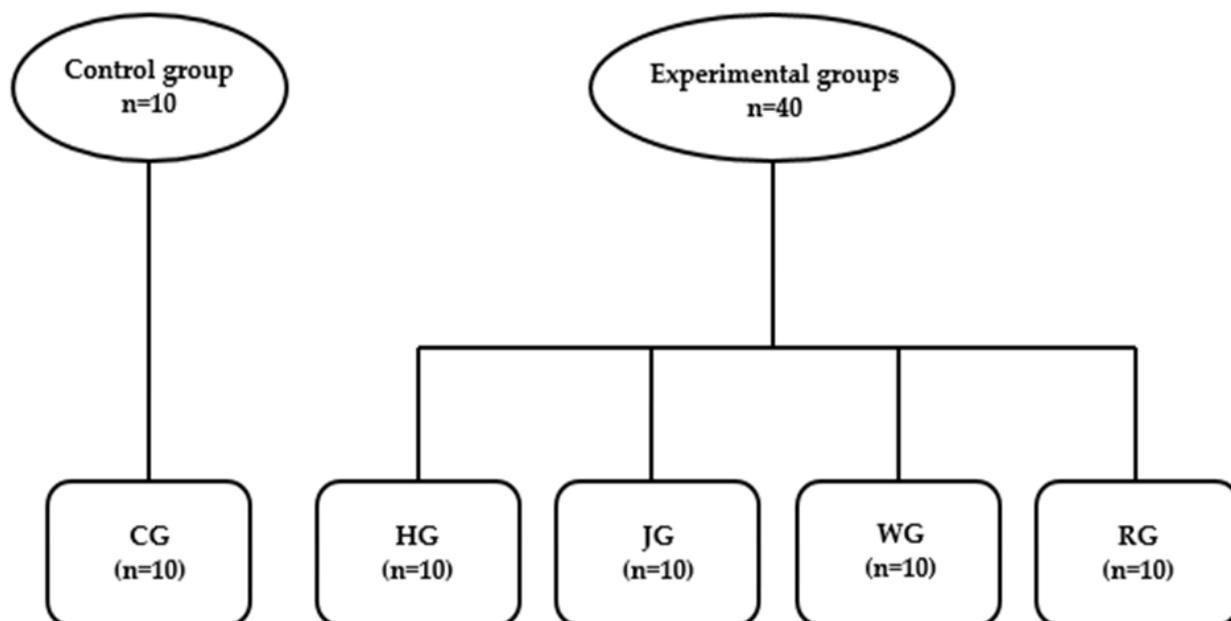

Figure 1. Dietary protocol. Experimental model: (CG) Control group supplemented with control feed and water; (HG) High-fat diet group received high-fat diet and water; (JG) Grape juice group represents animals submitted to high-fat diet treated with grape juice (15 mL/day) and water; (WG) Wine group represents animals submitted to high-fat diet treated with red wine (10 mL/day) and water; (RG) Resveratrol group represents animals submitted to high-fat diet treated with resveratrol solution isolated (15 mL/day - 40 mg/L) and water. Diets and solutions were administered during a period of 60 days.

juice, red wine and resveratrol solution) were measured daily by graduate beaker.

At the end of the experiment, the animals were exposed to vaginal smear to determine their stage of the estrous cycle and to ensure that there was no hormonal intervention in the research at the same physiological moment. Animals in estrus phase are isolated and after 6 hours of fasting anesthetized by intraperitoneal injection of a solution containing 11.50 mg/100 g ketamine body mass and 0.10 mg/100 g xylazine body mass and exsanguinated by cardiac puncture, according to Hem et al. (1998).

The hepatic tissue was carefully removed, weighed with a precision scale of BioPrecisa ® and the relative weight of the organ, denominated liver index, was calculated according to the equation: liver index = liver Weight (g) X 100/ body Weight (g). After weighing, four fragments of different lobes hepatics were sectioned with a surgical scalpel of each animal and immediately treated for cell cycle analysis, apoptosis assay and histological analysis (Guimarães et al. 2017).

Histological analysis

The livers were fixed in Bouin's solution for 24 hours and processed in graded of ethanol (70-100%) for 30 minutes each and washed twice in xylene for 15 minutes. Hepatic fragments were then individually embedded in paraffin, the blocks were cross-sected into 4μm thick slices, then stained with hematoxyl and eosin (HE). Photomicrographs were acquired and processed using a Zeiss ® (Oberkochen, Germany) Axioscop 20 microscope equipped with a Canon ® (Tokyo, Japan) G10 digital camera, a 14.7 megapixel JPG and an Image-Pro Plus ® software. Each slide had 10 analyzed fields looking at presence or absence of liver steatosis and portal inflammation, including leukocyte infiltrates in the portal zone. A total of 20 portal triads were evaluated in each slide and a percentage was determined by the number of portal triads containing (yes or not) leukocyte infiltration.

Table I. Group diets: control and high fat diets.

	CG	HG	JG	WG	RG
Casein*(g/100g ration)	14.0	14.0	14.0	14.0	14.0
Starch (g/100g ration)	62.1	46.07	46.07	46.07	46.07
Soy oil (mL/100g ration)	4.0	-	-	-	-
Lard (g/100g ration)	-	20.0	20.0	20.0	20.0
Cellulose (g/100g ration)	5.0	5.0	5.0	5.0	5.0
[1]Mix vitamins (g/100g ration)	1.0	1.0	1.0	1.0	1.0
[2]Mix minerals (g/100g ration)	3.5	3.5	3.5	3.5	3.5
B-choline (g/100g ration)	0.25	0.25	0.25	0.25	0.25
L-cystine (g/100g ration)	0.18	0.18	0.18	0.18	0.18
Sugar (g/100g ration)	10.0	10.0	10.0	10.0	10.0
Polyphenols (mg EAG/L)**			1.09	2.58	117.44
Trans-resveratrol mg/L)**			0.38	1.32	1.53

(*) % casein protein= 92.5% protein/100g casein; [(1)]Mix of vitamins (mg/kg ration): retinol palmitate 2.4, cholecalciferol 0.025, sodic bisulfite benadion 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6, thiamine hydrochloride 6.6 and tocopherol acetate 100.0; [(2)]Mix minerals (mg/kg ration): copper sulfate 0.1, ammonium molybdate 0.026, sodium iodate 0.0003, potassium chromate 0.028, zinc sulfate 0.091, calcium hydrogen phosphate 0.145, ammoniated iron sulfate 2.338, magnesium sulfate 3.37, manganese sulfate 1.125, sodium chloride 4.0, calcium carbonate 9.89 and potassium diidrogenophosfate 14.75; (**) search group data CG: Control group - control feed; HG: High-fat group; JG: Grape juice group; WG: Red wine group; RG: Resveratrol solution group; mg EAG: milligrams of gallic acid equivalents; mg: milligrams; L: liter.

Cell cycle analysis and cell viability

Flow cytometry analysis was performed to measure cell cycle and cell viability of tissue hepatic. The tissue was macerated and the extractions were made with the addition of 0.5 mg/ml collagenase (Sigma®). The cells were washed twice with phosphate buffered saline and resuspended in 500 µL of ice-cold Vindelov solution containing 0.1% Triton X-100, 0.1% citrate and 0.1 mg/mL of RNase and 50 mg/mL of propidium iodide (Sigma Chemical Co., St. Louis, MO) after centrifugation. After incubation for 15 min, the cell suspension was analyzed for DNA content by flow cytometry using a FACS Calibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). The relative proportions of cells with DNA content indicative of apoptosis (<2n), G_0/G_1 diploid (2n), S (2n < phase < 4n), and G_2/M phase (4n) were obtained and analyzed using the CellQuest WinMDI 2.9 program. Considering the experimental conditions that were used in this study, or in any others we were aware of, the fluorescence was not affected by the cell dissociation process. According to Guimarães et al. (2017), the nuclei of viable cells have been gated according to the relationship between FL-2W and FL2-A.

Detection of apoptosis by annexin V-FITC

To measure the rate of apoptosis, the cells were subjected to staining with Annexin V conjugated to FITC. The non-adherent cells were collected, and adherent cells were quickly washed with a calcium/magnesium-free buffered saline solution (BSS) and were detached with 0.125% trypsin/EDTA (Sigma Chemical Co., St. Louis, MO, USA) at room temperature. Subsequently, apoptotic and necrotic cells were stained with Annexin V FITC/Propidium Iodide (PI) (BD Pharmingen, Mountain View, CA,USA) according to the manufacturer's instructions, quantified with a flow cytometer (FACSCalibur, BD Bioscience,

Mountain View, CA, USA), and analyzed using two specific programs, Cell Quest and FlowJo.

Statistical analysis

The data were analyzed using the Windows Graphpad Prism software package. Differences between the groups were analyzed using the Student's t-test, and the values were considered unpaired and parametric. For means of comparison among the groups, analysis of variance (ANOVA one-way) and Tukey's post hoc test. The assumption of normality (Gaussian distribution) was verified by Kolmogorov-Smirnov tests to support the use of the statistical methods described above. Results are expressed as mean–standard deviation and the level of significance was set at p<0.05.

RESULTS

During experiment, the different dietary treatments did not affect (p>0.05) the intake of ration and water of animals which were similar among all groups. However, although no difference was observed in the hepatosomatic index, at the end of study the animals that consumed high-fat diet (HG, JG, WG and RG) showed higher (p<0.05) body weight (Table II). The consumption of RS and GJ was higher than the RW group, but despite the difference found in the consumption of beverages, all groups had similar energy consumption when we associated the feed consumption (Table II).

CG showed typical liver histology containing well-organized polygonal hepatocytes with eosinophilic cytoplasm and large central nuclei. Kupffer cells were found attached to endothelial cells in the sinusoids with elongated nuclei and compact chromatin (Figure 2a). On the other hand, the high-fat diet caused significant disruption in the liver architecture of all treated

Table II. Characteristics of dietary intake, bodyweight and hepatosomatic index of different groups.

Parameters	CG	HG	JG	WG	RG
Initial bodyweight (g)	215.22±38.15[a]	210.14±34.40[a]	217.56±31.60[a]	219.19±31.70[a]	227.20±25.27[a]
Final bodyweight (g)	226.40±20.88[a]	270.40±28.43[b]	267.60±17.00[b]	277.00±15.43[b]	278.00±16.83[b]
Liver index (g liver/ 100 g BW)	2.81±0.17[a]	2.73±0.12[a]	2.85±0.19[a]	2.66±0.26[a]	2.78±0.14[a]
Chow intake (g/100 g BW/day)	5.78±1.23[a]	5.15±1.16[a]	4.75±1.15[a]	4.70±1.06[a]	5.24±0.98[a]
Water intake (mL/100 g BW/day)	10.26±5.24[a]	10.06±6.39[a]	8.47±4.47[a]	6.65±2.28[a]	8.51±5.28[a]
Intake of beverages (mL/100 g BW/day)	-	-	3.87±1.49[a]	1.92±0.32[b]	5.34±0.94[c]
Total energy consumed (kcal/day)	48.24±5.31[a]	57.7±6.29[a]	57.5±14.05[a]	60.62±6.29[a]	57.28±5.07[a]

Values expressed as mean ± error standard of triplicate experiments. p value within same line. Different superscripts letters mean statistical difference between groups. Statistical significance was determined by ANOVA. followed by Tukey's post hoc multiple mean comparison test (p<0.05). CG: Control group - control feed; HG: High-fat group; JG: Grape juice group; WG: Red wine group; RG: Resveratrol solution group; mL: milliliter; g: grams; BW: bodyweight

groups (HG, JG, WG, RG). Rats of HG presented severe steatosis (Figure 2b) and the liver of JG was partially affected by high-fat diet protocol, indicating an intermediate score to steatosis (Figure 2c). The WG was similar to HG, indicating that wine treatment induced severe steatosis (Figure 2d). Interestingly, resveratrol protected the liver against damages induced by high-fat diet in 80% of mice (Figure 2e).

The high-fat diet results nonalcoholic steatohepatitis (NASH), which is well characterized by histological findings including liver steatosis and inflammation with hepatocyte injury. Here, high-fat diet (HG - Figure 3b and Figure 4) induced a severe liver inflammation marked by leukocyte infiltrate throughout the lobular and portal zones compared to control animals (CG - Figure 3a and Figure 4). Although JG presented portal triads with partial accumulation of extracellular matrix (Figure 3c and Figure 4), the JG showed significant decreases of NASH properties, such as less

lobular inflammation and steatosis than HG. WG presented histological findings compatible to HG, indicating that red wine maintains or aggravates the NASH (Figure 3d and Figure 4). RG showed significant reduction of high-fat diet damages. Resveratrol strongly inhibited the progression of NASH in these animals (Figure 3e and Figure 4).

Our results of cell cycle (Table III) and apoptosis (Table IV and Figure 5) are described below. High-fat diet (HG) altered the liver cell cycle of the animals leading to lower (p<0.05) number of viable cells and higher rate of apoptotic cells (Table IV) with an increase (p<0.05) in the number of cells in the G_2/M phase (Table III).

Grape juice (JG) was the only drink that reversed the effects of the high-fat diet on liver cells apoptosis. The animals of JG presented cell cycle and apoptosis rate of cells similar CG (Table IV and Figure 5). Even with a high-fat diet, the results show that grape juice protected the
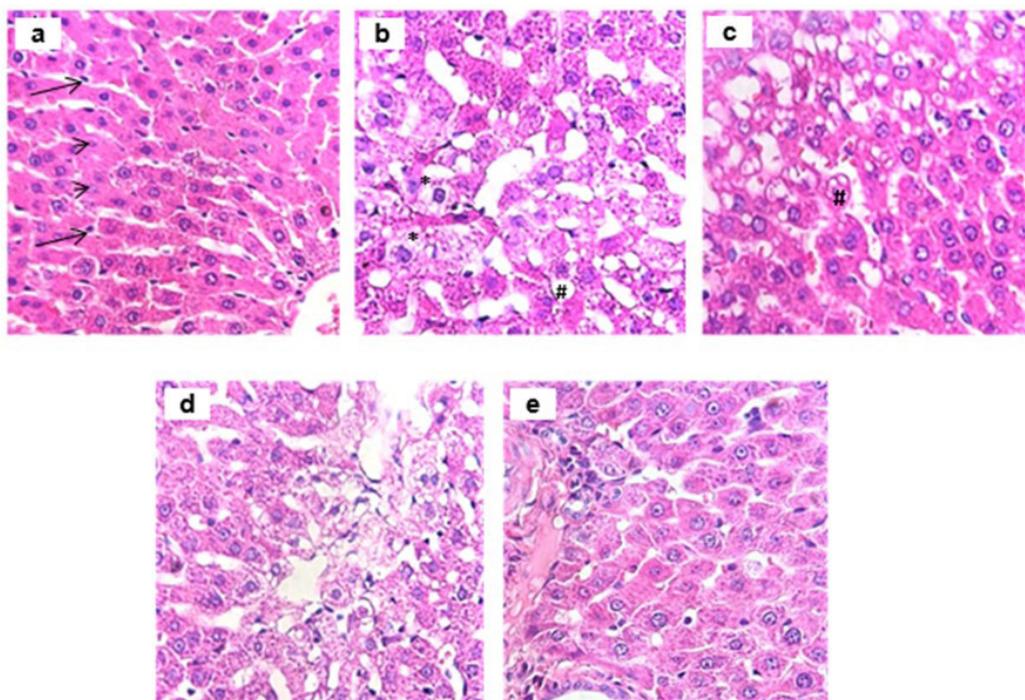
Figure 2. Photomicrographs of liver submitted to high-fat diet and distinct treatments. (a) Control group supplemented with control feed. (b) High-fat diet group showing severe steatosis. (c) Grape juice group represents animals submitted to high-fat diet treated with grape juice and partial steatosis in the liver. (d) Red wine group represents animals submitted to high-fat diet treated with red wine and high index of steatosis. (e) Resveratrol group represents animals submitted to high-fat diet treated with resveratrol and low index of liver injury. Hepatocytes (arrowhead); Kupffer Cells (arrow); Hepatocyte ballooning (*); Lipid accumulation (#). Data are representative of three experiments. Magnification: 400X. n=10 animals per group.

liver of the animal by maintaining unchanged percentage of viable cells, apoptotic cells and non-apoptotic cells (Table IV and Figure 5).

The wine consumption (WG) showed to be even more aggressive to the liver tissue. In addition to the alterations already caused by the high-fat diet, WG presented a reduction ($p<0.05$) in the number of cells in the G_0/G_1 phase (Table III), lower ($p<0.05$) viable cell rate and the highest ($p<0.05$) number of non-apoptotic cells (Table IV).

The animals that consumed resveratrol solution (RG) showed lower viable cell rate, increase of cells in the SubG1 phase and smaller number of G0/G1 and G2/M cells. The isolated compound induced programmed killing of the damaged cells and it promoted increase in the number of cells unsuitable for replication (SubG1) – Table III.

DISCUSSION

Most of the population consume a high-fat diet and the effects of relationship of diet and development of diseases are being evaluated (Fontelles et al. 2018, De Oliveira et al. 2016). Previous studies have been carried out on homeostatic tissues, such as the liver (Levy et al. 2011, Dani et al. 2007) and it has been shown that bioactive compounds present in grapes, such as resveratrol, promote hepatoprotective action against high-fat dietary intake (Buchner et al. 2014, Carpene et al. 2015, Farias et al. 2015). However, the source of consumption (juice, wine
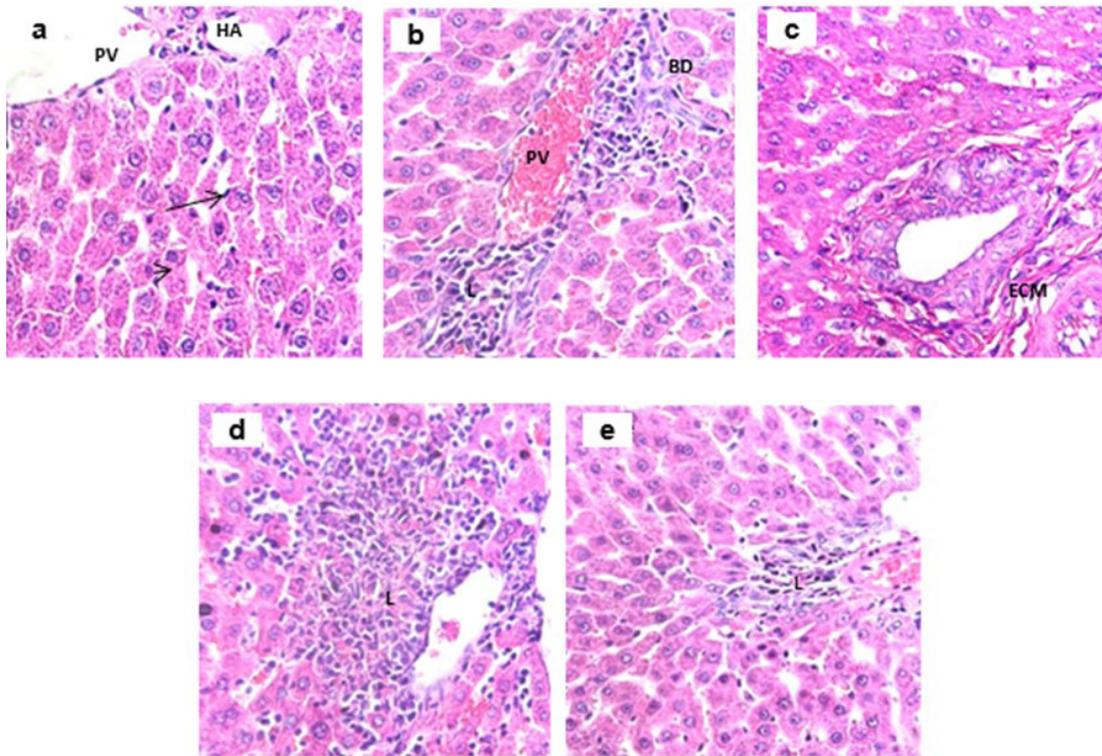
Figure 3. Photomicrographs of portal triads of liver submitted to high-fat diet. (a) Control group showing classical portal triad: branches of hepatic artery (HA), portal vein (PV) and bile ducts (BD); (b) High-fat diet group with significant portal leukocyte infiltrate; (c) Grape juice group reduced the NASH status, but extracellular matrix (ECM) deposition was found in some portal triads; (d) Red wine group with large leukocyte infiltration (L); (e) Resveratrol group reduced the leukocytes infiltrated in the portal triads. Hepatocytes (arrowhead); Kupffer Cells (arrow); Extracellular matrix deposition (ECM); Leukocytes (L). Data are representative of three experiments. Magnification: 400X. n=10 animals per group.
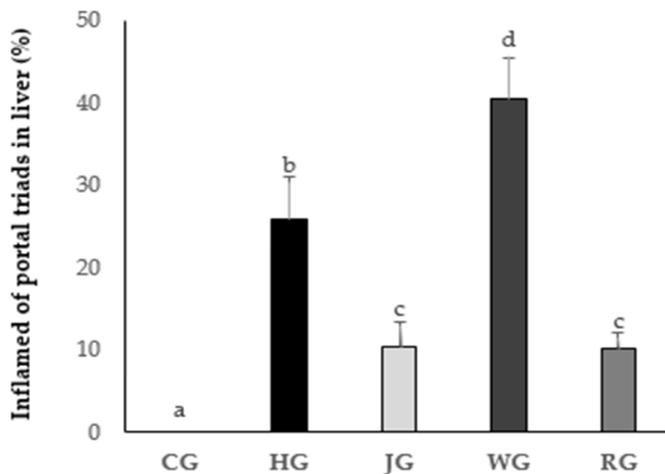

Figure 4. Effects of grape juice, red wine and resveratrol solution on inflamed of portal triads in liver of rats fed high-fat diet. Means significant difference with different letters (p<0.05). Abbreviations: (CG) Control group supplemented with control feed; (HG) High-fat diet group; (JG) Grape juice group represents animals submitted to high-fat diet treated with grape juice; (WG) Wine group represents animals submitted to high-fat diet treated with red wine; (RG) Resveratrol group represents animals submitted to high-fat diet treated with resveratrol solution isolated. Statistical significance was determined by ANOVA followed by Tukey's post hoc multiple mean comparison test.

Table III. Effect of grape juice, red wine and resveratrol solution on cell cycle progression of hepatic cells in rats fed high-fat diet.

Groups	SubG$_1$(%cells)	G$_0$/G$_1$(%cells)	S(%cells)	G$_2$/M(%cells)
CG	8.79±3.08[a]	61.05±0.63[a]	1.57±0.28[a]	16.42±7.62[a]
HG	7.13±1.91[a]	57.25±5.72[a]	2.92±0.93[b]	22.97±3.76[b]
JG	9.41±5.21[a]	63.17±18.81[a]	2.77±0.95[b]	12.93±4.87[a]
WG	8.00±1.37[a]	26.33±19.37[b]	2.81±0.79[b]	26.86±8.77[b]
RG	54.43±3.58[b]	24.05±16.17[b]	3.49±0.87[b]	7.26±1.32[c]

Different letters in the same attribute mean that samples are different (p<0.05) and same letters indicate the samples are the same (p>0.05). Abbreviations: (CG) Control group supplemented with control feed; (HG) High-fat diet group; (JG) Grape juice group represents animals submitted to high-fat diet treated with grape juice; (WG) Wine group represents animals submitted to high-fat diet treated with red wine; (RG) Resveratrol group represents animals submitted to high-fat diet treated with resveratrol solution isolated. Statistical significance was determined by ANOVA followed by Tukey's post hoc multiple mean comparison test.

Table IV. Effect of grape juice, red wine and resveratrol solution on stages of death process in hepatocytes of different groups (% cells).

Phases of Cell Death Process	CG	HG	JG	WG	RG
Viable cells (Annexin V⁻/PI⁻)	95.96±2.60[a]	85.36±4.21[b]	92.03±2.00[a]	80.81±15.81[b]	82.16±2.93[b]
Early apoptosis (Annexin V⁺/PI⁻)	1.23±0.81[a]	3.02±0.99[a]	1.30±0.85[a]	2.49±1.10[a]	2.01±1.23[a]
Late apoptosis (Annexin V⁺/PI⁺)	1.01±0.48[a]	8.61±3.51[b]	4.07±2.25[a]	7.77±3.82[b]	13.18±2.91[c]
Non-apoptotic death cells (Annexin V⁻/PI⁺)	3.01±1.36[a]	2.98±0.27[a]	2.87±1.45[a]	8.95±2.36[b]	3.00±0.39[a]

Results are expressed as the percentage of total cells. The data represent mean ± standard deviation values of triplicate experiments. Tukey test: different letters in the same phase of death process and extract means that samples are different (p<0.05) and same letters indicate samples are the same (p>0.05). Abbreviations: (CG) Control group supplemented with control feed; (HG) High-fat diet group; (JG) Grape juice group represents animals submitted to high-fat diet treated with grape juice; (WG) Wine group represents animals submitted to high-fat diet treated with red wine; (RG) Resveratrol group represents animals submitted to high-fat diet treated with resveratrol solution isolated. Viable cells (Annexin V⁻/PI⁻), Early apoptosis (Annexin V⁺/PI⁻), Late apoptosis/Necrosis (Annexin V⁺/PI⁺), Non-apoptotic cells (Annexin V⁻/PI⁺); PI- propidium iodide.

or compound isolated) has been fundamental for beneficial or deleterious effects to be observed when it comes to the association with high-fat diet (Martins & Nicoletti 2006, Cardozo et al. 2013, Dos Santos Lima et al. 2014, Reeves et al. 1993). Considering that the high-fat diet is a common choice of part of the population and that its deleterious effects have been observed (Fontelles et al. 2018, De Oliveira et al. 2016), this study aimed at assessing the effects of grape juice, red wine and isolated resveratrol on rat liver fed a high-fat diet, particularly with regard to the cell cycle, apoptosis and hepatocyte histology.

According to the recommendations of American Institute of Nutrition Recommendations for maintenance adult-rodents (AIN 93M), the daily consumption of lipids for adult rats should be 4% (Reeves et al. 1993). In our study, the diet contained 20% of total lipids, a concentration
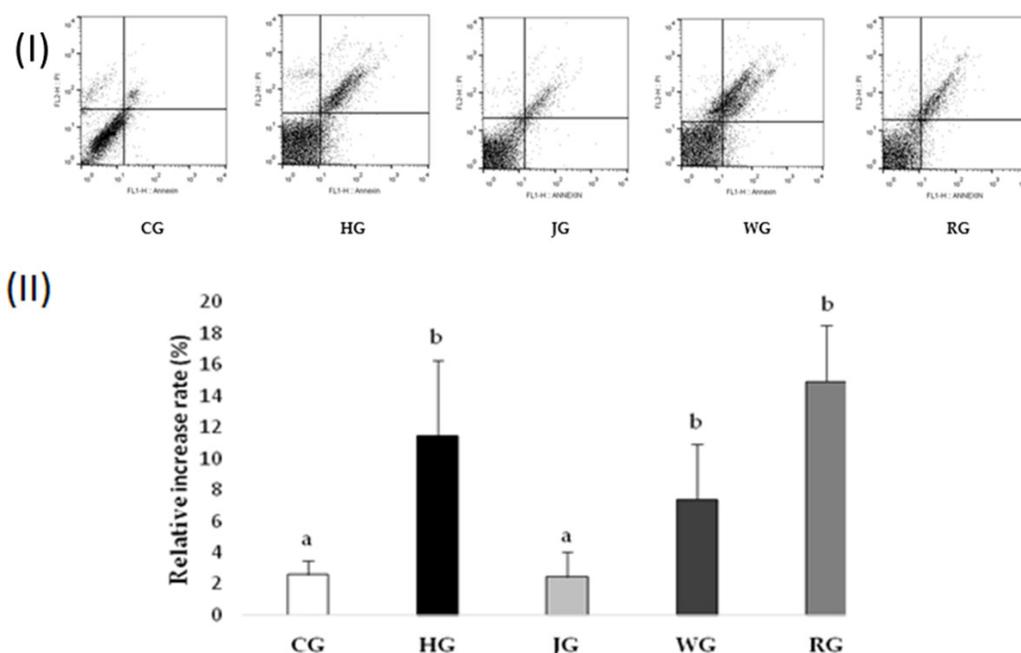
Figure 5. Effects of grape juice, red wine and resveratrol solution on rate apoptosis in rats fed high-fat diet. (I) Flow cytometry analysis of groups. (II) Relative increase rate of apoptotic cells of liver (%). Means significant difference with different letters (p<0.05). Abbreviations: (CG) Control group supplemented with control feed; (HG) High-fat diet group; (JG) Grape juice group represents animals submitted to high-fat diet treated with grape juice; (WG) Wine group represents animals submitted to high-fat diet treated with red wine; (RG) Resveratrol group represents animals submitted to high-fat diet treated with resveratrol solution isolated. Statistical significance was determined by ANOVA followed by Tukey's post hoc multiple mean comparison test.

five times higher than that recommended, which characterizes a high-fat diet (Wu et al. 2016). Studies show that the consumption of a diet with 20-50% of total lipids leads to weight gain and predisposes the individual to various diseases (El Ayed et al. 2018, Nolan et al. 2011, Gonçalves et al. 2018). This was also found in experiments with rats where there was a positive association between high-fat diet and animal body weight gain, even without higher caloric intake. (Martins & Nicoletti 2006, Nolan et al. 2011, Cheng et al. 2017, Soltis et al. 2017). These results corroborate our findings in which high-fat diet played a role in animal body weight gain (HG, JG, WG, RG). However, Gonçalves et al. (2018) demonstrated that pregnant rats treated with grape juice and high-fat diet presented lower weight gain when compared to the group that did not consume juice, evidencing that the consumption of grape

juice can minimize the gain of gestational bodyweight, as well as Buchner et al. (2014) and Cardozo et al. (2013) have stated. In this respect, it is worth mentioning that grape juice seems to have reduced the effects of the high-fat diet under bodyweight parameters, as the JG animals showed a trend of lower final weight as well as control animals that consumed a balanced diet.

No difference was found in the experiment between water and chow consumption with respect to the food consumption of the animals, although both were provided ad libitum. Our findings indicate that the animals accepted the manipulated diets well and that daily intake of feed was within the literature reported (Kim et al. 2011, Szkudelska et al. 2009). Although some studies affirm that there is a lower dietary intake of animals receiving a high-fat diet because they are able to regulate their intake

from the energy density of the diet consumed (Inglés et al. 2014, Meng et al. 2017), this effect may occur only in the short term, as there appears to be adaptation after a period of time and consequent normalization of dietary intake (Inglés et al. 2014). In our study, a trend of lower feed intake was observed in CG and JG. In long term, Kim et al. (2011) and Szkudelska et al. (2009) revealed that resveratrol in grape juice was able to decrease leptin secretion in rat adipocytes fed a high-fat diet, reducing their food intake justifying the trend of lower dietary intake of animals consuming grape juice in addition to high-fat diet.

Although the volume of grape juice (GJ), red wine (RW) and resveratrol solution (RS) is different, the doses of consumed beverages are in line with the literature. For whole red grape juice and red wine, the doses usually used range from 5 to 20 ml/day (Martins & Nicoletti 2017, Gu et al. 2016, Scott et al. 2012). The doses used for resveratrol alone show a significant variation, from 1 mg / day to 1.2 g resveratrol / day per animal (Buckton et al. 2018, Mukamal et al. 2016, Savage & Semple 2010, Charbonneau et al. 2007). The difference found in the consumption of drinks can be explained by the presence of refined sugar in SR and fructose in SU that make these drinks more tasty (Charbonneau et al. 2007), and the alcoholic content limits their intake in red wine (Deji et al. 2009).

The liver is a fundamental organ for the homeostasis of the organism, has a high metabolic rate and is very susceptible to any type of internal or external aggression, such as dietary, exercise or disease stressors (Lozano et al. 2016, Van et al. 2017). Studies in rats comparing the intake of a high-fat diet (66% lard) and with a balanced diet observed that the liver weight of the animals that consumed high fat did not differ from the control group (Carpene et al. 2015, Meng et al. 2017, Ohashi et al. 2018).

These findings corroborate the data from this study, since no difference was observed in the hepatosomatic index of the animals, regardless of the type of diet consumed. It should be noted, however, that the literature shows that the high consumption of saturated fatty acids is associated with inflammatory processes and lipotoxicity of certain organs, such as the liver, causing non-alcoholic fatty liver disease (NAFLD), which would be a possible dietary effect of our study (Carpene et al. 2015, Meng et al. 2017, Ohashi et al. 2018, Dixon et al. 2013, Alisi et al. 2017).

Although NASH frequently develops to progressive fibrosis, it is poorly observed in high-fat diet experimental condition (Eckert et al. 2015). Previous studies with animals have shown that high-fat diets induce accumulation of lipids in hepatocytes and Kupffer cells, triggering a process of inflammation (Seki & Schwabe 2015, Brunt et al. 1999, Festi et al. 2004, Santos et al. 2013, Farrell et al. 2012). Hepatic steatosis, once established, promotes cellular adaptations to high levels of oxidative stress, which would make the cells able to survive in this adverse environment, but would still keep the cells prone to the process of apoptosis and/ or necrosis associated with inflammation (Shono et al. 2011, Tunali-Akbay et al. 2010). Santos et al. (2013) also consider that excessive levels of fatty acids in hepatic tissue can cause damage to cellular proteins and lipids, increase oxidative stress and stimulate receptors associated with inflammatory hepatocellular lesions, defense cell activation, and tissue fibrosis. Our results corroborate these findings and resemble data where the high-fat diet altered the hepatocyte structure with ballooning degeneration and induced macrophage infiltration in rats (De Moura et al. 2016). Balloon degeneration is considered a form of hepatocyte death in the presence of steatosis and inflammation,

characterizing histopathological disorders via cell enlargement (Petyaev 2016). Excess dietary fat can also affect the immune system by activating pro-inflammatory Kupffer cells (M1 subtype) (Petyaev 2016). Kupffer cells are considered liver macrophages and their activation represents a central event in the initiation and progression of hepatic injury in in NASH-developed experimental models (Santos et al. 2013, Tunali-Akbay et al. 2010).

Petyaev et al. (2016) evaluated the effects of resveratrol alone and within the alcoholic matrix (red wine) on hepatocytes attacked by UV-B radiation as a model of oxidative stress and obtained results contrary to ours. The authors concluded that resveratrol showed synergistic antioxidant effect in wine in both lower and higher dosages to make the other compounds more effective than alone. Another study evaluated trans-resveratrol, red wine and deionized wine supplementation and showed that the three treatments altered the biomarkers of oxidative stress generated by the high-fat diet, but had no effect on the prevention or regression of fat accumulation in the liver (Cheng et al. 2017). However, more recent studies corroborate our findings when stating that resveratrol alone promotes therapeutic effects against fat infiltration in the liver, induces lower levels of resveratrol, inflammation and modulates the immune system by activating Kupffer cells with phagocytic activity (Chassot et al. 2018, Liu et al. 2016). Our findings with grape juice corroborate De Moura et al. (2016) which also found a hepatoprotective effect in grape juice, concluding that juice consumption was able to prevent tissue degeneration in the liver.

Several studies investigate the effects of isolated compounds on cellular parameters and apoptosis to determine your mechanisms of action in tissues. In this sense, however, few studies have investigated the effect of combinations of phytochemicals and diets (Dudley et al. 2011, Fausto et al. 2006).

Tissue homeostasis is maintained in multicellular organisms by the balance between cell proliferation and death of cells (Mitchell & Willenbring 2008). Cell cycle is the process by which genetic material within a cell is replicated and secreted into two new cell compartments, characterizing tissue growth and proliferation (Krysko et al. 2008). In contrast to many cell types, hepatocytes are known to maintain constant replication and rejuvenation of their cells (Tarantino 2007, Estrov et al. 2003). However, external factors can interfere in the process of adequate cell proliferation, such as dietary factors, and the defective or inefficient elimination of altered cells may contribute to the development of different pathologies. In this regard, apoptosis and non-apoptotic death represent a continuous process of equilibrium between proliferation and cell death that are required for tissue homeostasis when they occur in a controlled manner (Viola & Soehnlein 2015).

Cell changes induced by consumption of a high-fat diet include apoptosis that is considered a common mechanism of hepatic injury and an important point of NAFLD (Yuzefovych et al. 2013, Simsa-Maziel & Monsonego-Ornan 2012, Han et al. 1995, Chiang et al. 2014). These effects may be linked to liver NAFLD genesis, such as NASH. In this regard, it is essential to identify dietary strategies that can contribute to its prevention and reduction.

The hypothesis is that the excess fat in the diet promoted tissue damage and programmed death of the injured cells. There was a possible attempt of cell proliferation to compensate for the higher rate apoptotic cells, but the cellular changes generated by the diet were identified and induced cell cycle arrest (Koo 2013). Any change in the apoptosis process is deleterious and results in tissue damage, which

can be generated by dietary inadequacies. Chiang et al. (2014) found that the expression of apoptosis and fibrosis had been altered by mice receiving a high protein diet. In excessive fat consumption, fatty acid oxidation and oxidative phosphorylation for adenosine triphosphate (ATP) production are increased. Deregulation of this pathway results in energy deficiencies and/or production of reactive oxygen species responsible for cell damage (Dudley et al. 2011, Valenzuela et al. 2018, Jung et al. 2006). This was also evidenced in the study by Dudley et al. (2011) who reported that consumption of high-fat diet during pregnancy and lactation resulted in epigenetic modifications, with DNA methylation, associated with compromised regulation of cell cycle genes in the offspring's liver during early postnatal life.

In other studies, the consumption of grape juice had already been protective to the cell cycle of tissues. According study of De Moura et al. (2016), grape juice prevented changes in the cycle of rat cells liver attacked by cadmium, avoiding tissue degeneration. Previous works showed that grape juice prevented cell proliferation and consequent progression of the disease in cancer cells models (De Moura et al. 2006, Kong et al. 2019).

The data suggest that wine consumption inhibited cell growth and that alcoholic consumption has induced non-apoptotic cell death as an additional internal aggressive agent (Ghantous et al. 2018, Araim et al. 2002, Singh et al. 2017). Normal cells only proliferate in response to cellular development or signs of mitosis that indicate tissue growth, while proliferation of altered cells, such as cancer cells, occurs uncontrolled by stimulus induction internal and/or external aggressors (Bianchini & Vainio 2003). These data may be related to the alcohol content of the beverage, as alcohol results in epigenetic changes in the cell cycle

and reduces cell viability, as previous studies have shown. (Jesus et al. 2017, Wang et al. 2017). Furthermore, alcohol was responsible for the disordered increase in cell proliferation of the oral mucosa of wistar rats exposed to various resveratrol presentations in the study by Jesus et al. (2017).

Regarding resveratrol, some studies have indicated that the compound would offer benefits in preventing or neutralizing cell damage, aging, cancer, and other cell cycle related diseases (Alarcon de La Lastra & Villegas 2005, Oliveira et al. 2009). However, more recent work has shown that the isolated resveratrol inhibits the enzymatic activity of the two cyclooxygenase forms, leading to cell cycle arrest, G_0/G_1 phase cell deprivation, inhibition of G_2/M cell progression and apoptosis induction (Ghantous et al. 2018, Araim et al. 2002, Singh et al. 2017). This is consistent with what was found in our analysis when we observed that isolated resveratrol appears to have been cytototoxic, induced the programmed killing of the damaged cells, and promoted an increase in the number of cells unfit to replicate.

CONCLUSIONS

The high-fat diet promoted damage to the liver tissue, altering the cell cycle and histology of the liver. In addition, the diet rich in fat infiltration of liver cells, inflammation and infiltration of leukocytes in the tissue. Excess dietary fat reduced the contents of viable cells and increased apoptosis, even when combined with intake of wine or resveratrol alone. Red wine aggravated the histological damages caused by the high-fat diet, but these lesions were reduced by the intake of grape juice and resveratrol alone. On the other hand, grape juice protected the liver from diet-generated cell damage, showing

the cell cycle profile and the viability compatible with the control that consumed a balanced diet.

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