beverages

MDPI

Article

Grape Juice Consumption with or without High Fat Diet during Pregnancy Reduced the Weight Gain and Improved Lipid Profile and Oxidative Stress Levels in Liver and Serum from Wistar Rats

Luciana Kneib Gonçalves, Gabrielli Bortolato, Ruben Dario Braccini Neto, Marina Rocha Frusciante, Claudia Funchal and Caroline Dani *

Laboratório de Bioquímica do Centro Universitário Metodista IPA, Cel. Joaquim Pedro Salgado, 80, Porto Alegre 90420-060, RS, Brazil; l.kneib@yahoo.com.br (L.K.G.); gabriellibortolato@yahoo.com.br (G.B.); rubenbraccini@gmail.com (R.D.B.N.); marina_frusciante@hotmail.com (M.R.F.); csfunchal@yahoo.com.br (C.F.)
* Correspondence: carolinedani@yahoo.com.br; Tel.: +55-51-3316-1298

Received: 30 August 2018; Accepted: 10 October 2018; Published: 21 October 2018

Abstract: The aim of this study was to evaluate the effects of high fat diet with or without grape juice during the pregnancy on gestational weight gain, biochemical parameters, and oxidative stress in plasma and liver from Wistar rats. Forty-nine rats were divided into four groups: control diet group (CD), high fat diet (HFD), grape juice and control diet (PGJCD), and grape juice and high fat diet (PGJHFD). During the treatment the weight gain of the rats was tracked. They had free access to their respective diets during 42 days of treatment. After offspring weaning, the mother rats were euthanized and blood and liver were collected. The high fat diet increased the total cholesterol and triglycerides serum levels as well as carbonyl levels in the liver, however this diet reduced the high-density lipoprotein (HDL) and urea levels in serum. Grape juice consumption reduced gestational body weight gain. In liver, the juice consumption increased sulfhydryl levels and reduced the superoxide dismutase (SOD) activity and TBARS level, in serum the consumption reduced aspartate aminotransferase (AST) and TBARS. We can conclude that the consumption of a diet rich in fat can promotes harmful effects on health during pregnancy, however the consumption of grape juice seems to be an important alternative to prevent oxidative damages and to promote the improvement of health.

Keywords: antioxidants; gestational model; obesity; polyphenols

1. Introduction

Pregnancy is a process that causes various changes in the maternal physiological state, since this is necessary due to the greater demand for nutrients essential for proper fetal development [1]. A hyperlipidic diet is defined by the above average fat intake of 30 to 45% of the total daily calories [2]. There is a relationship already evidenced in epidemiological studies, as well as in the animal model, that the consumption of a diet rich before and during gestation promoted the development of metabolic disorders, such as adipogenesis, stimulation of chronic inflammation, and oxidative cellular imbalance, factors associated with gestational obesity [3,4]. In addition, excess weight gain during pregnancy may be associated with gestational diabetes, birth difficulties, and even health risks to the fetus during the perinatal period into adult life [5]. Recently studies showed that paternal and maternal obesity increase the chance of breast cancer in female offspring [6–8].

As a consequence of these and other modifications, pregnant women, mainly obese ones, are more exposed to deleterious actions that free radicals (FR) and/or reactive species (RE) can cause in an

organism [9]. This process known as oxidative stress (OE) is established when the antioxidant defenses are not equivalent or sufficient in relation to the reactive species and their substrates [10]. Still, according to the literature, EO has been frequently related to the etiology of pathological processes that affects the reproductive process [11,12]. In view of this, the consumption of grape derivatives such as grape juice can be an alternative to combat OE due to the presence of nutrients and bioactive compounds with antioxidant action in their composition [13–15].

As previously mentioned, among the metabolic and biochemical changes that may be present during gestation, obesity, diabetes, and liver disorders are the ones with the highest incidence in this population [1,5,12]. Pregnancy cholestasis is a heterogeneous disease specific for pregnancy characterized by hepatic dysfunction and intense pruritus [16]. Among the main laboratory findings reported are the plasma increase of hepatic transaminases [17]. In addition to causing harm to maternal health, it is also linked to an increase in fetal morbidity and mortality, and medium-risk perinatal complications such as preterm birth to more severe complications such as fetal uterine death [16–18].

Studies from our group have already proven the hepatoprotective effects after the consumption of this beverage [15,19], one of those studies demonstrated that grape juice intake was able to decrease fat accumulation in hepatocytes in a high fat diet model [19]. In addition, a recent study by Wohlenberg et al. (2015) demonstrated that grape juice consumption during the pregnancy and lactation time promoted an antioxidant and hepatoprotective effects in Wistar rats [20].

Maternal obesity is becoming an increasing public health issue, and it is known that nutrition and metabolism play a crucial role in the health and well-being of both mother and fetus [21]. Considering the high fat diet is a common choice in this population, and deleterious effects were already observed [7,22], associated with the findings about the health benefits from grape juice consumption, this study aims to evaluate the gestational weight gain, biochemical profile, and parameters of EO in the serum and liver of Wistar rats. At the time of writing this paper, there were no other studies in the literature demonstrating the possible protective effects that grape juice consumption could promote against the deleterious effect from high fat diet during pregnancy.

2. Methodology

2.1. Grape Juice

Purple grape juice *Vitis labrusca* L. variety Bordo was kindly provided by Fante drinks. The juice was from the harvest of 2015 and all from the same lot.

2.2. Analysis of Phenolic Compounds

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 m pore Nylon membranes. The total content of phenolic compounds of the grape juices were measured using the colorimetric modification of Folin-Ciocalteau method, as described by Singleton, (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 analysis of phenolic compounds was performed on an HP model 1100 HPLC, 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 5min, 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 Morelli (2011). 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. Diets

The nutritional composition from conventional and high fat diet are presented at Table 1. The high-fat diet was acquired ready and its composition was provided by the manufacturer as follows: corn starch (14.95%), casein (20.00%), dextrinized starch (10.00%), saccharose (10.00%), lard (31.00%), soy oil (4.00%), microcrystalline cellulose (5.00%), L-cystine (0.30%), choline bitartrate (0.25%), BHT (0.0010%), mineral mix ain 93 g (3.50%), and vitamin mix (1.00%). The diet was purchased from Pragsoluções Biosciences (Jau, Sao Paulo, SP, Brazil) and this information was provided by this company.

Table 1. Nutritional composition at two different diets, conventional and high fat diet (100 g).

Content	Conventional Diet		High Fat Diet	
	Calories	Gram	Calories	Gram
Carbohydrates	240	66	100	25
Protein	88	22	80	20
Lipids	36	4	450	50
Total	364		630	

2.4. Animals

Initially, we used 78 Wistar female and 39 male Wistar rats, 12 weeks old and weighing approximately 290 g from the bioterium of the IPA Methodist University. These animals were placed in a ratio of 2 mating females for each one (2:1), had free access to their respective diets, and were maintained in a light–dark cycle of ±12 h at a temperature of 22 °C ± 1 °C. The entire experiment was conducted with the approval of the Ethics Committee on Animal Use (ECAU) of the Methodist University Center-IPA (number 019/2014 e 009/2015).

2.5. Treatment

During pregnancy (approximately 21 days) and lactation (approximately 21 days), damns were divided into 4 groups: control diet (CD), high-fat diet (HFD), control diet and purple grape juice (PGJCD), and purple grape juice and high-fat diet (PGJHFD). Dams had free access to water and feed according to the diet of the defined group. The pregnant rats had free access to water and diet, according to the group. All groups received water, however the purple grape juice groups received the juice plus. The daily consumption of grape juice was of approximately 72.02 ± 31.94 mL (PGJCD) and 48.51 ± 23.14 mL (PGJHFD). Also during treatment, the gestational weight gain of these animals was monitored 3 times a week with a digital scale balance (Crystal 200, Gibertini, Italy). After lactation, the animals were euthanized by decapitation. Trunk blood was collected which was centrifuged to obtain the serum and stored at about 0 °C. The liver of these animals was also removed, homogenized in 1.5% KCl, and refrigerated at −20 °C until the moment of analysis.

2.6. Biochemical Parameters Evaluated in Serum

Total cholesterol (TC) (mg/dL), triglycerides (TG) (mg/dL), and HDL (mg/dL) were used as biochemical markers for assessing lipid profile. Liver function was analyzed using alanine aminotransferase (ALT) (U/L) and aspartate aminotransferase (AST) (U/L). Urea (mg/dL) and creatinine (mg/dL) were used as kidney function markers. Assays were performed in serum by automation (BioclinBS120).

2.7. Oxidative Stress

Oxidative stress parameters were evaluated in serum and liver. The test that evaluates reactive substances to hiobarbituric acid (TBARS) was used to measure levels of lipid peroxidation, as previously described by Wills (1996). [23]. TBARS was determined by absorbance at 535 nm. The results were expressed in nmol/mg protein. Oxidative damage to proteins was measured by determining the carbonyl groups and it is based on the reaction with dinitrophenylhydrazine (DNPH) according to Levine et al. (1990) [24]. The results were expressed in nmol/mg protein. The non-enzymatic defenses were determined by sulfhydryl technique. This assay is based on the reduction of 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) by thiol groups, yielding a yellow compound (TNB) which has its absorbance determined with a spectrophotometer at 412 nm [25]. The sulfhydryl content is inversely correlated to the protein oxidative damage. The results were expressed in nmol/mg protein.

The activity of superoxide dismutase (SOD) was determined with a spectrophotometer by measuring the inhibition of adenocromo autocatalytic formation rate at 480 nm (SP-2200 Spectrophotometer, Bioespectro Curitiba, Brazil) in a reaction environment containing 1mM adrenaline and 50 mM glycine [26]. The results were expressed as U SOD/mg protein. Catalase activity (CAT) was assessed according to the method described by Aebi (1984), which determines the rate of decomposition of H2O2 at 240 nm (SP-2200 Spectrophotometer, Bioespectro). The results were expressed as U CAT/mg protein [27].

2.8. Protein Determination

Protein concentration was determined according to the method described by Lowry et al. (1951) [28].

2.9. Statistical Analysis

Results were expressed as mean and standard error of the mean, and the normality of the data was assessed by the Kolmogorov-Smirnov test, checking normal distribution of data. Differences between groups were analyzed using ANOVA two-way (factor group and factor handling), followed by post Holm-Sidak-test, with $p < 0.05$ considered significant. All analyses were performed using the statistical softwares Statistical Package for Social Sciences (SPSS) version 17.0 (International Business Machines Corporation, New York, NY, USA) and SigmaStat (Jandel Scientific Software, San Jose, CA, USA).

3. Results

The total phenolic compounds of grapevine *Vitis labrusca* L. were quantified by Folin-Ciocalteau and the majority compounds were identified by high-performance chromatography (HPLC) with pure standards of resveratrol, catechin, epicatechin, naringin, gallic acid, chlorogenic acid, and ferulic acid (Table 2).

Table 2. Total phenolic content and majority compounds in purple grape juice by HPLC.

Phenolic Compounds (mg/L)	Average	Standard Deviation
Total phenolic compounds	2796.57	11.20
Total flavonoids content	77.27	2.10
Resveratrol	0.506	0.01
Epicatechin	1.95	0.04
Naringin	3.37	0.11
Rutin	17.41	0.25
Chlorogenic acid	12.37	0.12

We monitored weight gain during pregnancy and observed a significant difference in measures eight ($p = 0.031$), nine ($p = 0.006$), ten ($p < 0.001$), and eleven ($p < 0.001$), corresponding respectively to 16, 18, 24, and 26 days of treatment, where the groups consuming grapes presented lower weight than the

control groups, and the PGJCD group had the lowest weight among all the related groups (Figure 1). Also, when evaluating the gestational weight gain (final weight–initial weight), we observed that the beverage factor promoted a reduction in gestational weight gain, where the groups that consumed the juice presented values lower than the control groups (Figure 2).

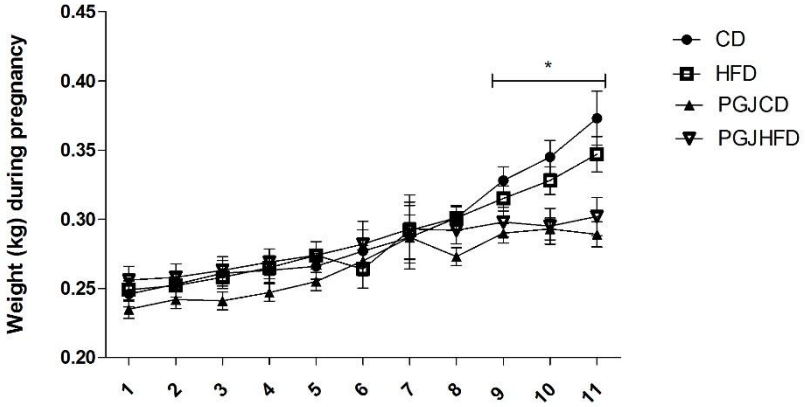

Figure 1. Weight evaluation during the pregnancy in rats from different groups (kg). * Statistically differences by two-ways ANOVA, pos hoc Holm-Sidak ($p < 0.\ 05$). CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

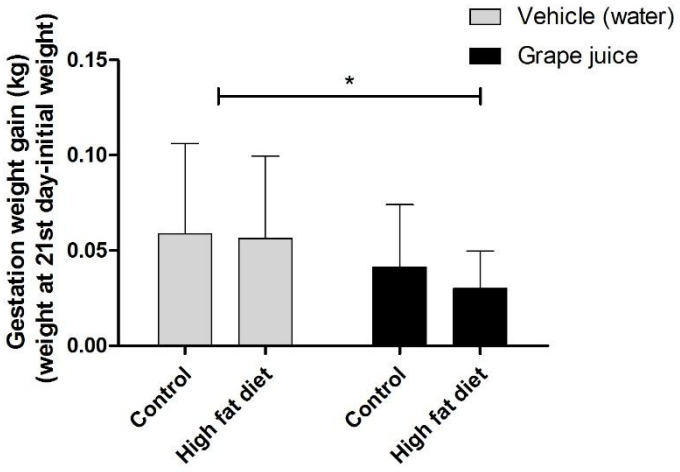

Figure 2. Weight gain during pregnancy (kg) in rats from different groups (kg). * Statistically differences by two-ways ANOVA, pos hoc Holm-Sidak ($p < 0.\ 05$). CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

We observed that the diet was determinant for the alterations in lipidic profile. CT and TG have increased levels, and HDL levels have been reduced in dams who consumed a high fat diet with or without grape juice consumption (Table 3). In order to evaluate hepatic function through AST level, we observed statistically differences in type of drink (water = 266.54 $\pm$ 8.670, juice = 234.28 $\pm$ 10.50) and with the diet factor (control = 265.69 $\pm$ 9.16, high fat diet = 235.12 $\pm$ 10.10). The rats from PGJCD and PGJHFD groups showed the lowest levels if comparing with other groups (Table 3). The high fat diet was the factor that caused increases in ALT level (Table 3). As for renal markers, the dams who consumed a high fat diet during the gestation reduced urea level (Table 3), no differences in serum creatinine levels were observed (Table 3).

Table 3. Biochemistry parameters in serum of female Wistar rats (n = 49) treated with high fat diet or control diet, and with or without grape juice during pregnancy and lactation times ($p < 0.05$).

Parameters	Group			
	CD	HFD	PGJCD	PGJHFD
Total cholesterol (mg/dL)	69.43 ± 3.87	81.67 ± 4.17 *	69.80 ± 4.57	97.33 ± 5.90 *
Triglycerides (mg/dL)	65.20 ± 10.59	115.67 ± 9.66 *	79.75 ± 11.83	138.67 ± 13.67 *
HDL (mg/dL)	39.71 ± 2.33	26.67 ± 2.52 *	36.80 ± 2.76	33.50 ± 3.08 *
AST (U/L)	268.57 ± 11.82	264.50 ± 12.77	262.80 ± 13.99	205.75 ± 15.64 *,#
ALT (U/L)	115.43 ± 6.69	74.83 ± 7.43 *	123.80 ± 8.14	69.00 ± 9.10 *
Urea (mg/dL)	79.00 ± 3.30	44.50 ± 3.56 *	75.80 ± 3.90	44.25 ± 4.36 *
Creatinine (mg/dL)	0.46 ± 0.030	047 ± 0.03	0.52 ± 0.03	0.52 ± 0.04

Values expressed in mean ± EPM. * Statistically differences ($p < 0.05$) by two-way ANOVA with Holm-Sidak pos hoc from the control (CD). # $p < 0.05$ vs. HFD. HDL: High Density Lipoprotein; Alanine transaminase (ALT); Aspartate transaminase (AST); CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

Regarding lipid peroxidation levels, we observed that the beverage consumption alters this parameter in liver and serum. In serum, the females from grape juice group had lower TBARS levels (2.57 ± 0.44) than the non-consumers (3.69 ± 0.42) (Figure 3A). In hepatic tissue, the lower lipid damages were also observed in rats consumed the grape juice during pregnancy (2.57 ± 0.21) when compared to the non-consumers rats (3.44 ± 0.24) (Figure 3B).

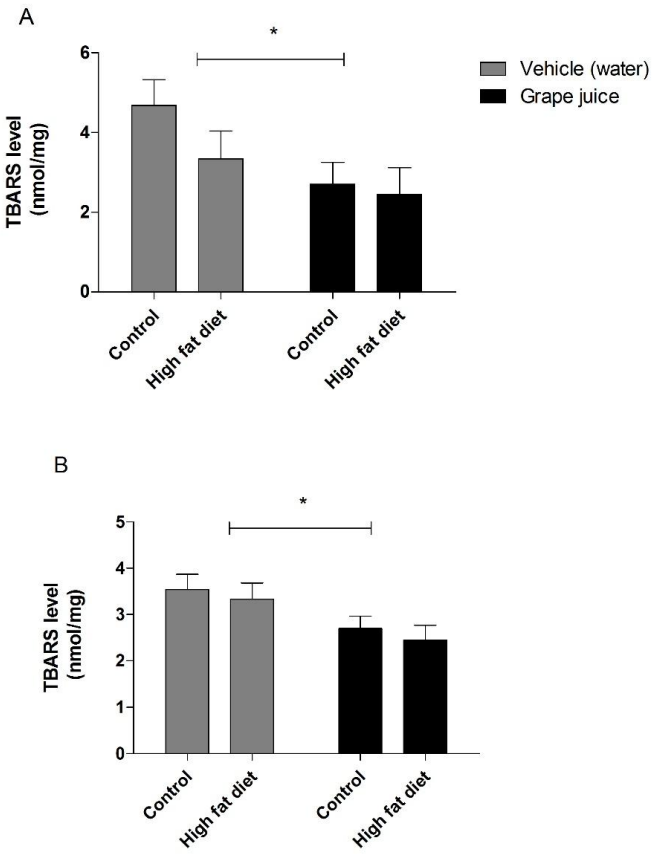

Figure 3. Levels of TBARS (nmol/mg of protein) in serum (**A**) and liver (**B**) in female rats after the lactation period from different groups. The results are expressed as mean ± SEM. * Statistical difference according to ANOVA two-way, followed by post-test Holm-Sidak ($p < 0.05$). CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

Regarding protein oxidation (carbonyl levels), no significant differences in serum were observed between the treated groups ($p > 0.05$) (Figure 4A). When evaluated in the liver, we found that mothers who consumed a high fat diet had higher protein damage in the liver (107.29 ± 13.37) than the control group (60.72 ± 11.37) (Figure 4B).

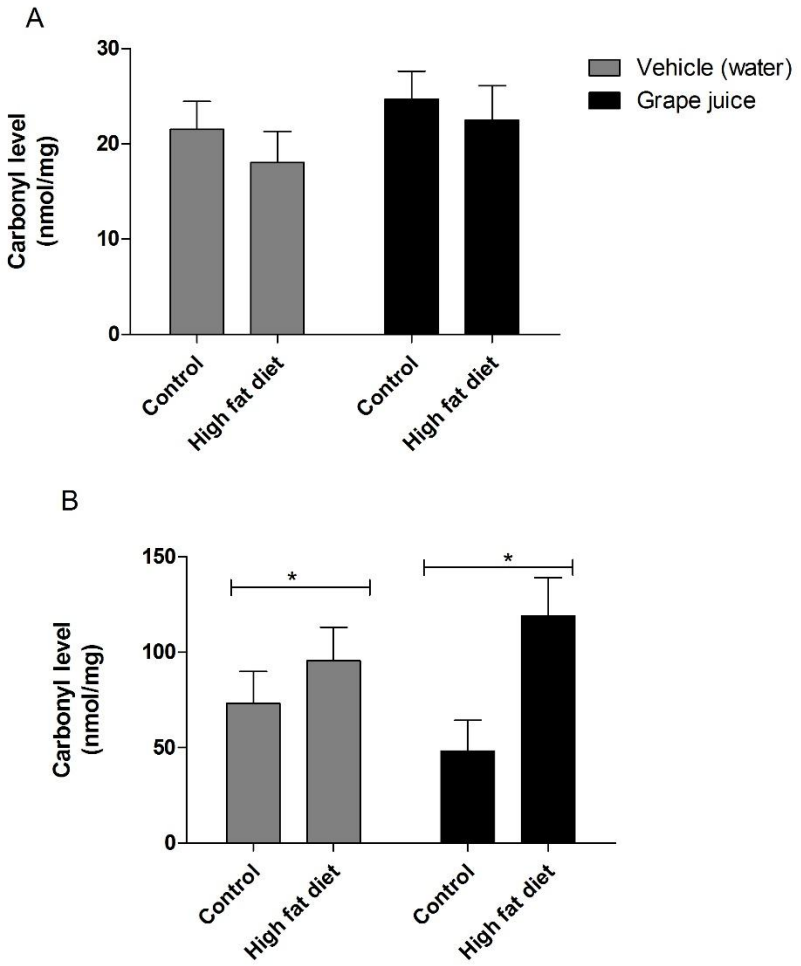

Figure 4. Levels of Carbonyl (nmol/mg of protein) in serum (**A**) and liver (**B**) in female rats after the lactation period from different groups. The results are expressed as mean ± SEM. * Statistical difference according to ANOVA two-way, followed by post-test Holm-Sidak ($p < 0.05$). CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

We did not observe difference in the factors interactions (beverages and diets) in plasma thiols (sulfhydryl) levels ($p > 0.05$) (Figure 5A). On the other hand, dams who consumed grape juice during pregnancy presented increased levels of these groups in the liver (30.39 ± 1.29) when compared to the control groups (21.88 ± 1.43) (Figure 5B). As for antioxidant activity, we observed that grape juice consumption had a lower SOD activity in the liver (22.81 ± 9.07) compared to than the non-grape juice consumers (57.80 ± 9.67) (Table 4). In CAT activity no significant differences were found in the liver among the groups of dams ($p > 0.05$) (Table 4).

Table 4. Determination of antioxidant enzymes activity in liver of Wistar dams (n = 49) treated with control or high fat diet and with or without grape juice during pregnancy and lactation periods ($p < 0.05$).

Enzymes Actvity	C	HFD	PGJCD	PGJHDF
SOD (U SOD/mg)	45.45 ± 13.35	70.13 ± 14.00	23.25 ± 12.28 *	22.38 ± 13.35 *
CAT (U CAT/mg)	3.95 ± 0.76	2.91 ± 0.84	3.29 ± 0.66	3.07 ± 0.80

Values expressed in mean ± EPM. * Statistically differences ($p < 0.05$) by two-way ANOVA with Holm-Sidak pos hoc. CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

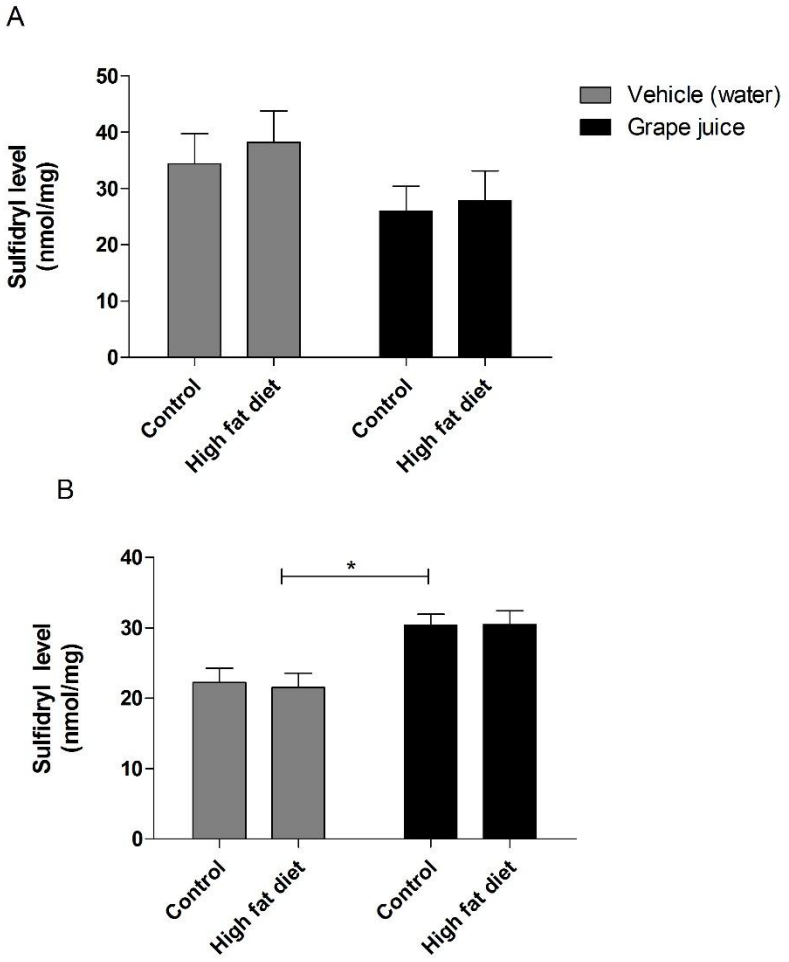

Figure 5. Levels of Sulfidryl (nmol/mg of protein) in serum (**A**) and liver (**B**) in female rats after the lactation period from different groups. Results are expressed as mean ± SEM. * Statistical difference according to ANOVA two-way, followed by post-test Holm-Sidak ($p < 0.05$). CD: control diet; HFD: high fat diet; PGJCD: purple grape juice and control diet; PGJHFD: purple grape juice and high fat diet.

4. Discussion

Our study aimed to evaluate the influence of treatment with a high fat diet and grape juice (*Vitis labrusca* L.) consumption on gestational weight gain, biochemical parameters, and oxidative stress in the serum and liver. Maternal nutrition is of extreme importance to fetus health and to guarantee adequate gestational development, since the nutritional source of the fetus is exclusively derived from the mother's alimentary ingestion [21]. Excessive weight gain during pregnancy has been associated with the development of gestational diabetes, labor difficulties, and risk to the fetus in the perinatal period [5].

Our results showed that mothers who consumed grape juice during pregnancy had lower weight gain when compared to mothers in the control groups. Some studies in the literature show that resveratrol, a compound found in grape juice, was able to decrease the adipocyte leptin secretion in vitro and in vivo in rats fed a high fat diet, reducing food intake [29,30]. In addition, corroborating our results, studies from our group demonstrated that adult rats treated with grape juice associated with a high fat diet presented lower body weight gain when compared to the control group also submitted to the same type of diet, thus evidencing the benefits that the consumption of grape juice can bring about the reduction of gestational body weight gain [19,31].

The analysis of biochemical parameters enables us to detect stress conditions, which can be caused by multiple factors [32]. Through the results of our experiment, we verified that diet was the decisive factor for the changes observed in the lipid profile, due to the increase in the TC, TG, and the reduction of the HDL from mothers who received a high fat diet associated or not with juice consumption of grape. Previous studies, in animal models, have also shown these same deleterious effects on the lipid profile as a consequence of the intake of a high-fat diet observed in our experiment [33–35]. Although the consumption of grape juice during gestation did not reduce the damages caused by the high fat diet on lipid profile, studies with grape derivatives have already shown a positive influence on these parameters [36,37]. In a study with rodents submitted to a hypercholesterolemic diet and treated with a fraction of non-alcoholic ethyl acetate (EAF) of a wine produced from V. labrusca L. grapes, reductions in CT and TG levels were observed [36]. Also, another experiment with hyperglycemic hamsters treated with non-alcoholic red wine evidenced a reduction in the CT and LDL levels of these animals [37]. Still, several studies have demonstrated significant increases in HDL after treatment with different types of grape juice [38–40].

The AST and ALT markers were used to evaluate the hepatic function of pregnant rats. Both factors (drink and diet) influenced AST enzyme alterations. However, the lowest values were observed in grape juice consumers groups. Our results are in accordance with a study with grape juice from the Turkish variety *Vitis vinifera*. In this study the consumption was able to reduce the increase of AST caused by CCl4, emphasizing the relevant beneficial results of the juice on this enzyme [41]. The diet factor altered ALT levels, and grape juice consumption was not able to prevent the significant reduction of this enzyme caused by the high fat diet. A similar result was also observed in a study with alcoholic extract of V. vinifera leaf in its (n-BuOH) fraction; in that study the extract decreased the levels of ALT and AST [42]. However, in a study involving a diabetic rat model, *Vitis labrusca* L. grape leaf extract did not cause significant changes in ALT [43].

Regarding renal function, a high fat diet, with or without grape juice, reduced urea levels. Changes in urea caused by the consumption of a high-fat diet were also observed in other studies, but in these studies the phenolic compounds were able to revert the damage caused by diet, restoring urea levels to normal [44,45]. Still regarding the renal profile, in our study there were no significant differences regarding creatinine levels. The same was observed in a study conducted with grape seed extract, where it did not reduce this parameter in Wistar rats with renal injury induced by gentamicin [46].

Because of the physiological changes caused by gestation, there is a higher consumption of O_2 as well as changes in the consumption of energy substrates, resulting in a greater exposure to OE [47]. Not only the OE have been contributing to the development of diseases, but high fat diet consumption has been a strong influence on the etiology of diseases [22,48,49]. Lipid peroxidation and protein oxidation in serum and liver of Wistar rats after gestation and lactation were measured, and we observed that the mothers who consumed grape juice during pregnancy (PGJCD and PGJHFD) showed lower levels of lipoperoxidation than non-consumers. These results are similar to a study with organic and conventional grape juice, in which the groups that consumed the juice had lipid peroxidation reduced in the liver of rats submitted to a high fat diet [19]. Also corroborating with our results, the levels of lipid peroxidation were reduced in the plasma of pregnant sheep in a study using diet supplemented with polyphenols (51). Furthermore, a clinical study with subjects who consumed grape juice for two weeks demonstrated the reduction of TBARS in serum and plasma (52).

According to Barakauskas et al. [50] in a study about the effects of sub-chronic clozapine and haloperidol administration on brain lipid level, the lipid peroxidation may be influenced by availability of peripheral lipids. This is accordance with our study. We observed that the groups with the highest cholesterol level showed the highest lipid peroxidation level. The high-fat diet (HFD) and the positive energy balance result in a large stock of triacylglycerol (TG) in the adipose cells, especially in the visceral fat, leading to adipocyte hypertrophy [51]. This dysfunctional (hypertrophic) adipocyte may lose its ability to store more lipids, leading to ectopic fat accumulation in other tissues such as the liver. It has been postulated that increased adiposity in obesity are key mediators of oxidative stress that may play a causal role in multiple forms of obesity-associated complications such as insulin resistance and type 2 diabetes [52]. According to da Costa et al. [51] it is important to identify new strategies that can effectively address obesity-related complications such as hyperglycemia, inflammation, and oxidative stress as well as reduce the risk of obesity-associated diseases. These authors showed that polyphenol-rich extract from Vitis vinifera L. grape skin prevented the oxidative stress and inflammation in liver and adipose tissue.

In this sense, previous studies have already shown alterations in protein oxidation provoked by organic and conventional purple grape juice consumption, with a reduction in protein oxidation levels in plasma and serum Wistar rats [15,20,53]. In the liver, we verified that the intake of grape juice was not able to reduce the levels of protein oxidation elevated by a high fat diet. In contrast, another study from our group showed that a high fat diet, despite increasing lipid peroxidation, did not affect the protein oxidation in the liver of rats [19]. Furthermore, it has been demonstrated grape juice has hepatoprotection effects where it was able to reduce the liver damage caused by different drugs [53]. When evaluating the protein damage in the serum of the mothers, no significant differences were observed. This difference between liver and serum response could be explain because the liver is more sustainable in this model with high fat diet, according to HFD feeding caused inflammation and oxidative stress in the liver of rats [54].

As previously mentioned, the amount of sulfhydryl groups is inversely correlated with protein damage [25]. At the serum level, in our experiment no significant differences were observed between groups on the quantification of thiol grouping levels. Similar findings were observed in a study by Wohlenberg (2015), where gestational consumption of grape juice did not cause changes in non-enzymatic defense [20]. However, grape juice consumption increased this parameter level in the liver. Several studies with grape derivatives have demonstrated the ability of these products to increase non-enzymatic antioxidant defenses against OE damage in both serum and liver of rats [19,43,53].

Among the body's primary antioxidant defense lines, we can highlight the SOD and CAT enzymes [55]. Among the groups evaluated, the grape juice consumption reduced the SOD activity in the hepatic tissue, whereas no significant differences were observed in the levels of CAT enzymatic activity. A previous study of our group had already observed the reduction of SOD in the group of mothers who received grape juice throughout gestation via gavage. Also, during this same experiment no differences were observed in CAT activity in liver [20]. On the other hand, supplementation with grape juice became effective reversed the decrease in SOD activity impaired by the use of radiation in the liver of rodents [56]. Also, unlike our findings, there was an increase in CAT activity in the liver of animals that consumed organic grape juice [15] and, in contrast to this, another study demonstrated a reduction of this enzyme in the hepatic tissue of rodents that received a single dose of PTZ [53].

5. Conclusions

We can observe the importance of maternal nutritional choices. Consumption of grape juice can reduce weight gain during pregnancy, ensuring the health of the mother and the development of her offspring. Also, the juice was effective mainly on serum levels of AST and on lipid peroxidation, reducing these damages in both serum and liver and promoting the increase of non-enzymatic defenses in the liver tissue. These findings, together with those in the literature, reinforce the protective effect of grape juice in relation to OE. In spite of this, more studies are needed to better elucidate and understand

the primary and secondary mechanisms that may be involved in this process, including the possible transgenerational effects on the health of offspring in the short and long term.

Author Contributions: L.K.G., C.F. and C.D. conceived/designed the experiments and contributed reagents/materials; L.K.G., M.R.F., R.D.B.N., G.B. and C.D. performed the fieldwork; K.G., M.R.F., R.D.B.N. and G.B. carried out analytical determinations; L.K.G. and C.D. analyzed the data; L.K.G., C.F. and C.D. discussed the results; L.K.G. and C.D. wrote the manuscript and. discussed and revised the paper.

Funding: This research was funded by IBRAVIN and Centro Universitário Metodista IPA.

Acknowledgments: Centro Universitário Metodista PA, CAPES, IBRAVIN, FAPERGS and CNPq.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Souza, A.I. De Nutrição em obstetrícia e pediatria. *Rev. Bras. Saúde Matern. Infant.* **2004**, *4*, 203–204. [CrossRef]
2. Kennedy, E.; Bowman, S.; Powell, R. Dietary-fat intake in the US population. *J. Am. Coll. Nutr.* **1999**, *18*, 207–212. [CrossRef] [PubMed]
3. Lee, S.; Kwak, H.-B. Role of adiponectin in metabolic and cardiovascular disease. *J. Exerc. Rehabil.* **2014**, *10*, 54–59. [CrossRef] [PubMed]
4. Kruse, M.; Seki, Y.; Vuguin, P.M.; Du, X.Q.; Fiallo, A.; Glenn, A.S.; Singer, S.; Breuhahn, K.; Katz, E.B.; Charron, M.J. High-Fat Intake During Pregnancy and Lactation Exacerbates High-Fat Diet-Induced Complications in Male Offspring in Mice. *Endocrinology* **2013**, *154*, 3565–3576. [CrossRef] [PubMed]
5. Ziegler, E.E.; Filer, L.J. Conocimientos actuales sobre nutrición. *Rev. Esp. Salud Publica* **1998**, *72*, 379–380. [CrossRef]
6. Fontelles, C.C.; Guido, L.N.; Rosim, M.P.; Andrade, F.O.; Jin, L.; Inchauspe, J.; Pires, V.C.; de Castro, I.A.; Hilakivi-Clarke, L.; de Assis, S.; et al. Paternal programming of breast cancer risk in daughters in a rat model: Opposing effects of animal- and plant-based high-fat diets. *Breast Cancer Res.* **2016**, *18*, 71. [CrossRef] [PubMed]
7. Fontelles, C.C.; da Cruz, R.S.; Hilakivi-Clarke, L.; de Assis, S.; Ong, T.P. Investigation of Paternal Programming of Breast Cancer Risk in Female Offspring in Rodent Models. In *Methods in Molecular Biology (Clifton, N.J.)*; Humana Press: New York, NY, USA, 2018; Volume 1735, pp. 207–220.
8. Nguyen, N.M.; de Oliveira Andrade, F.; Jin, L.; Zhang, X.; Macon, M.; Cruz, M.I.; Benitez, C.; Wehrenberg, B.; Yin, C.; Wang, X.; et al. Maternal intake of high n-6 polyunsaturated fatty acid diet during pregnancy causes transgenerational increase in mammary cancer risk in mice. *Breast Cancer Res.* **2017**, *19*, 77. [CrossRef] [PubMed]
9. Agarwal, A.; Allamaneni, S.S.R. Role of free radicals in female reproductive diseases and assisted reproduction. *Reprod. Biomed. Online* **2004**, *9*, 338–347. [CrossRef]
10. Markesbery, W.R. The Role of Oxidative Stress in Alzheimer Disease. *Arch. Neurol.* **1999**, *56*, 1449–1452. [CrossRef] [PubMed]
11. Agarwal, A.; Gupta, S.; Sikka, S. The role of free radicals and antioxidants in reproduction. *Curr. Opin. Obstet. Gynecol.* **2006**, *18*, 325–332. [CrossRef] [PubMed]
12. Saleh, M.M.; Abdo, K.R. Intrahepatic cholestasis of pregnancy: Review of the literature and evaluation of current evidence. *J. Womens Health (Larchmt)* **2007**, *16*, 833–841. [CrossRef] [PubMed]
13. Dani, C.; Oliboni, L.S.; Vanderlinde, R.; Bonatto, D.; Salvador, M.; Henriques, J.A.P. Phenolic content and antioxidant activities of white and redred juices manufactured with organically- or conventionally-produced grapes. *Food Chem. Toxicol.* **2007**, *45*, 2574–2580. [CrossRef] [PubMed]
14. Dani, C.; Pasquali, M.A.B.; Oliveira, M.R.; Umezu, F.M.; Salvador, M.; Henriques, J.A.P.; Moreira, J.C.F. Protective Effects of RedPurple grape Juice on Carbon Tetrachloride-Induced Oxidative Stress in Brains of Adult Wistar Rats. *J. Med. Food* **2008**, *11*, 55–61. [CrossRef] [PubMed]
15. Dani, C.; Oliboni, L.S.; Pasquali, M.A.B.; Oliveira, M.R.; Umezu, F.M.; Salvador, M.; Moreira, J.C.F.; Henriques, J.A.P. Intake of RedPurple grape Juice as a Hepatoprotective Agent in Wistar Rats. *J. Med. Food* **2008**, *11*, 127–132. [CrossRef] [PubMed]

16. Saleh, M.M.; Abdo, K.R. Intrahepatic Cholestasis of Pregnancy: Review of the Literature and Evaluation of Current Evidence. *J. Womens Health* **2007**, *16*, 833–841. [CrossRef] [PubMed]
17. Arrese, M.; Reyes, H. Intrahepatic cholestasis of pregnancy: A past and present riddle. *Ann. Hepatol.* **2006**, *5*, 202–205. [PubMed]
18. Wasmuth, H.E.; Glantz, A.; Keppeler, H.; Simon, E.; Bartz, C.; Rath, W.; Mattsson, L.-A.; Marschall, H.-U.; Lammert, F. Intrahepatic cholestasis of pregnancy: The severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. *Gut* **2007**, *56*, 265–270. [CrossRef] [PubMed]
19. Buchner, I.; Medeiros, N.; Lacerda, D.D.S.; Normann, C.A.B.M.; Gemelli, T.; Rigon, P.; Wannmacher, C.M.D.; Henriques, J.A.P.; Dani, C.; Funchal, C. Hepatoprotective and Antioxidant Potential of Organic and Conventional Grape Juices in Rats Fed a High-Fat Diet. *Antioxidants* **2014**, *3*, 323–338. [CrossRef] [PubMed]
20. Farias, M.; Gonçalves, L.K.; Schaffer, T.K.; Hilger, D.K.; Dario, R.; Neto, B.; Antunes, C.; Frusciante, M.; Rodrigues, A.; Funchal, C.; et al. Effect of grape juice on some biochemical and oxidative stress parameters in serum and liver enzymes of pregnant and lactating rats. *Issues Biol. Sci. Pharm. Res.* **2015**, *3*, 37–46. [CrossRef]
21. Marchi, J.; Berg, M.; Dencker, A.; Olander, E.K.; Begley, C. Risks associated with obesity in pregnancy, for the mother and baby: A systematic review of reviews. *Obes. Rev.* **2015**, *16*, 621–638. [CrossRef] [PubMed]
22. De Oliveira Andrade, F.; Fontelles, C.C.; Rosim, M.P.; de Oliveira, T.F.; de Melo Loureiro, A.P.; Mancini-Filho, J.; Rogero, M.M.; Moreno, F.S.; de Assis, S.; et al. Exposure to lard-based high-fat diet during fetal and lactation periods modifies breast cancer susceptibility in adulthood in rats. *J. Nutr. Biochem.* **2014**, *25*, 613–622. [CrossRef] [PubMed]
23. Wills, E.D. Mechanisms of lipid peroxide formation in animal tissues. *Biochem. J.* **1966**, *99*, 667–676. [CrossRef] [PubMed]
24. Levine, R.L.; Garland, D.; Oliver, C.N.; Amici, A.; Climent, I.; Lenz, A.G.; Ahn, B.W.; Shaltiel, S.; Stadtman, E.R. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* **1990**, *186*, 464–478. [PubMed]
25. Aksenov, M.Y.; Markesbery, W.R. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. *Neurosci. Lett.* **2001**, *302*, 141–145. [CrossRef]
26. Bannister, J.V.; Calabrese, L. Assays for superoxide dismutase. *Methods Biochem. Anal.* **1987**, *32*, 279–312. [PubMed]
27. Aebi, H. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121–126. [PubMed]
28. Lowry, O.H.; Rosebrouh, N.J.; Lewis-Farr, A.L.; Randall, R.J. Protein Measurement with the Folin phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef] [PubMed]
29. Kim, S.; Jin, Y.; Choi, Y.; Park, T. Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice. *Biochem. Pharmacol.* **2011**, *81*, 1343–1351. [CrossRef] [PubMed]
30. Szkudelska, K.; Nogowski, L.; Szkudelski, T. The inhibitory effect of resveratrol on leptin secretion from rat adipocytes. *Eur. J. Clin. Investig.* **2009**, *39*, 899–905. [CrossRef] [PubMed]
31. Cardozo, M.G.; Medeiros, N.; Dos Santos Lacerda, D.; De Almeida, D.C.; Henriques, J.A.P.; Dani, C.; Funchal, C. Effect of chronic treatment with conventional and organic redpurple grape juices (*Vitis labrusca*) on rats fed with high-fat diet. *Cell. Mol. Neurobiol.* **2013**, *33*, 1123–1133. [CrossRef] [PubMed]
32. Aderimi, F. Effects of Replacement of Wheat Bran with Cassava Root Sieviate Supplemented or Unsupplemented with Enzyme on Hematology and Serum Biochemistry of Pulled Chicks. *J. Trop. For. Sci.* **2004**, *7*, 147–153.
33. Shankar, K.; Singh, S.; Kumar, D.; Varshney, S.; Gupta, A.; Rajan, S.; Srivastava, A.; Beg, M.; Srivastava, A.; Kanojiya, S.; et al. *Cucumis melo* ssp. *Agrestis var. Agrestis* Ameliorates High Fat Diet Induced Dyslipidemia in Syrian Golden Hamsters and Inhibits Adipogenesis in 3T3-L1 Adipocytes. *Pharmacogn. Mag.* **2015**, *11*, 501–510. [CrossRef]
34. Eleftheriades, M.; Pervanidou, P.; Vafaei, H.; Vaggos, G.; Dontas, I.; Skenderi, K.; Sebire, N.J.; Nicolaides, K. Metabolic profiles of adult Wistar rats in relation to prenatal and postnatal nutritional manipulation: The role of birthweight. *Hormones (Athens)* **2014**, *13*, 268–279. [CrossRef] [PubMed]
35. Han, Y.; Lin, M.; Wang, X.; Guo, K.; Wang, S.; Sun, M.; Wang, J.; Han, X.; Fu, T.; Hu, Y.; et al. Basis of aggravated hepatic lipid metabolism by chronic stress in high-fat diet-fed rat. *Endocrine* **2015**, *48*, 483–492. [CrossRef] [PubMed]

36. Hort, M.A.; Schuldt, E.Z.; Bet, Â.C.; DalBó, S.; Siqueira, J.M.; Ianssen, C.; Abatepaulo, F.; de Souza, H.P.; Veleirinho, B.; Maraschin, M. Anti-Atherogenic Effects of a Phenol-Rich Fraction from Brazilian Red Wine (*Vitis labrusca* L.) in Hypercholesterolemic Low-Density Lipoprotein Receptor Knockout Mice. *J. Med. Food* **2012**, *15*, 936–944. [CrossRef] [PubMed]
37. Vinson, J.A.; Teufel, K.; Wu, N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis* **2001**, *156*, 67–72. [CrossRef]
38. ZibaeeNezhad, M.J.; Mohammadi, E.; Babaie Beigi, M.A.; Mirzamohammadi, F.; Salehi, O. The Effects of Unripe Grape Juice on Lipid Profile Improvement. *Cholesterol* **2012**, *2012*, 890262. [CrossRef] [PubMed]
39. Khadem-Ansari, M.H.; Rasmi, Y.; Ramezani, F. Effects of purple grape juice consumption on high density lipoprotein-cholesterol, apolipoprotein AI, apolipoprotein B and homocysteine in healthy human volunteers. *Open Biochem. J.* **2010**, *4*, 96–99. [CrossRef] [PubMed]
40. Castilla, P.; Echarri, R.; Dávalos, A.; Cerrato, F.; Ortega, H.; Teruel, J.L.; Lucas, M.F.; Gómez-Coronado, D.; Ortuño, J.; Lasunción, M.A. Concentrated purple grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am. J. Clin. Nutr.* **2006**, *84*, 252–262. [CrossRef] [PubMed]
41. Pirinççioğlu, M.; Kızıl, G.; Kızıl, M.; Özdemir, G.; Kanay, Z.; Aydın Ketani, M. Protective effect of Öküzgözü (*Vitis vinifera* L. cv.) grape juice against carbon tetrachloride induced oxidative stress in rats. *Food Funct.* **2012**, *3*, 668–773. [CrossRef] [PubMed]
42. Orhan, D.D.; Orhan, N.; Ergun, E.; Ergun, F. Hepatoprotective effect of *Vitis vinifera* L. leaves on carbon tetrachloride-induced acute liver damage in rats. *J. Ethnopharmacol.* **2007**, *112*, 145–151. [CrossRef] [PubMed]
43. Lacerda, D.S.; Santos, C.F.; Oliveira, A.S.; Zimmermann, R.; Schneider, R.; Agostini, F.; Dani, C.; Funchal, C.; Gomez, R. Antioxidant and hepatoprotective effects of an organic grapevine leaf (*Vitis labrusca* L.) extract in diabetic rats. *RSC Adv.* **2014**, *4*, 52611–52619. [CrossRef]
44. Ahad, A.; Ahsan, H.; Mujeeb, M.; Siddiqui, W.A. Gallic acid ameliorates renal functions by inhibiting the activation of p38 MAPK in experimentally induced type 2 diabetic rats and cultured rat proximal tubular epithelial cells. *Chem. Biol. Interact.* **2015**, *240*, 292–303. [CrossRef] [PubMed]
45. Zhou, Y.; Lin, S.; Zhang, L.; Li, Y. Resveratrol prevents renal lipotoxicity in high-fat diet-treated mouse model through regulating PPAR-α pathway. *Mol. Cell. Biochem.* **2016**, *411*, 143–150. [CrossRef] [PubMed]
46. Safa, J.; Argani, H.; Bastani, B.; Nezami, N.; Rahimi Ardebili, B.; Ghorbanihaghjo, A.; Kalagheichi, H.; Amirfirouzi, A.; Mesgari, M.; Soleimany Rad, J. Protective effect of grape seed extract on gentamicin-induced acute kidney injury. *Iran. J. Kidney Dis.* **2010**, *4*, 285–291. [PubMed]
47. Vannucchi, C.I.; Jordao, A.A.; Vannucchi, H. Antioxidant compounds and oxidative stress in female dogs during pregnancy. *Res. Vet. Sci.* **2007**, *83*, 188–193. [CrossRef] [PubMed]
48. Moses, G.S.; Jensen, M.D.; Lue, L.-F.; Walker, D.G.; Sun, A.Y.; Simonyi, A.; Sun, G.Y. Secretory PLA 2-IIA: A new inflammatory factor for Alzheimer's disease. *J. Neuroinflammation* **2006**, *3*. [CrossRef] [PubMed]
49. Puglielli, L. Aging of the brain, neurotrophin signaling, and Alzheimer's disease: Is IGF1-R the common culprit? *Neurobiol. Aging* **2008**, *29*, 795–811. [CrossRef] [PubMed]
50. Barakauskas, V.E.; Ypsilanti, A.R.; Barr, A.M.; Innis, S.M.; Honer, W.G.; Beasley, C.L. Effects of sub-chronic clozapine and haloperidol administration on brain lipid levels. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2010**, *34*, 669–673. [CrossRef] [PubMed]
51. Da Costa, G.F.; Santos, I.B.; de Bem, G.F.; Cordeiro, V.S.C.; da Costa, C.A.; de Carvalho, L.C.R.M.; Ognibene, D.T.; Resende, A.C.; de Moura, R.S. The Beneficial Effect of Anthocyanidin-Rich *Vitis vinifera* L. Grape Skin Extract on Metabolic Changes Induced by High-Fat Diet in Mice Involves Antiinflammatory and Antioxidant Actions. *Phyther. Res.* **2017**, *31*, 1621–1632. [CrossRef] [PubMed]
52. Van Greevenbroek, M.M.J.; Schalkwijk, C.G.; Stehouwer, C.D.A. Dysfunctional adipose tissue and low-grade inflammation in the management of the metabolic syndrome: Current practices and future advances. *F1000Research* **2016**, *5*, 2515. [CrossRef] [PubMed]
53. Rodrigues, A.D.; Scheffel, T.B.; Scola, G.; dos Santos, M.T.; Fank, B.; Dani, C.; Vanderlinde, R.; Henriques, J.A.P.; Coitinho, A.S.; Salvador, M. RedPurple grape juices prevent pentylenetetrazol-induced oxidative damage in the liver and serum of Wistar rats. *Nutr. Res.* **2013**, *33*, 120–125. [CrossRef] [PubMed]
54. Delwing-de Lima, D.; Ulbricht, A.S.S.F.; Werlang-Coelho, C.; Delwing-Dal Magro, D.; Joaquim, V.H.A.; Salamaia, E.M.; de Quevedo, S.R.; Desordi, L. Effects of two aerobic exercise training protocols on parameters of oxidative stress in the blood and liver of obese rats. *J. Physiol. Sci.* **2018**, *68*, 699–706. [CrossRef] [PubMed]

55. Halliwell, B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? *Lancet* **1994**, *344*, 721–724. [CrossRef]
56. Andrade, E.R.; Cruz, I.B.M.; Andrade, V.V.R.; Piccoli, J.C.E.; González-Gallego, J.; Barrio, J.P.; González, P. Evaluation of the potential protective effects of ad libitum black grape juice against liver oxidative damage in whole-body acute X-irradiated rats. *Food Chem. Toxicol.* **2011**, *49*, 1026–1032. [CrossRef] [PubMed]