Original Research Article

Effect of grape juice on some biochemical and oxidative stress parameters in serum and liver enzymes of pregnant and lactating rats

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This study aimed to evaluate biochemical and oxidative stress parameters in serum and liver of Wistar rats, in the period after pregnancy and lactation, treated chronically with red grape juice variety Bordo (Vitis labrusca). Forty Wistar rats were placed for mating, and the confirmation of pregnancy was through vaginal swab. After, these animals were randomly divided into 4 groups with and without handling. The groups submitted to handling were treated by gavage (7µL/g) and groups not submitted to handling were treated by free access. Results on this study show that red grape juice reduced the levels of aspartate aminotransferase (liver enzyme) in the serum of the handled animals tested (p<0.05). Regarding the parameters of oxidative stress, red grape juice was able to reduce the levels of protein oxidation in liver and serum of the animals as well as to modulate the enzymatic activity of superoxide dismutase in the liver and the enzymatic activity of catalase in the serum of the same, consequently promoting changes in the relation of these enzymes (p<0.05). Based on the obtained results, it is possible to see the red grape juice V. labrusca benefits presented against the evaluated biochemical and oxidative stress parameters, suggesting it as an important ally in the diet of pregnant women.

Key words: Biochemical markers, hepatoprotection, pregnancy

INTRODUCTION

Among the abnormalities that can occur during pregnancy, it is known the development of a disease characterized by liver dysfunction and severe pruritus named as cholestasis of pregnancy (Feitosa et al., 2009). This disease can present maternal effects, however, is more associated with the increased of fetal morbidity and mortality and also with the increase in perinatal risk as uterine death and preterm delivery (Feitosa et al., 2009).

The literature considers greater exposure to oxidative stress as a major factor responsible for the onset or development of pathological processes that affect the female reproductive process (Agarwal and Allamaneni, 2004; Agarwal, Gupta and Sikka, 2006; Vannuchi, Jordan and Vannuchi, 2006). Oxidative stress is established when antioxidant defenses are not proportional or effective in the face of reactive species and their products (Agarwal, Gupta and Sikka, 2006; Markesberry, 1999).

Among the alternatives with potential antioxidant effect, is the grape juice. This product made from Vitis labrusca grapes has many nutrients and bioactive compounds with antioxidant and antimutagenic action as well as anticancer activity (Dani et al., 2007; Dani et al., 2008a; Dani et al., 2008b). It stands out because it does not have alcohol, allowing it to be consumed by most people, including children, pregnant women and even patients with hepatitis.

It is known that the grape juice, through its antioxidant action, is able to prevent damage to the liver (Dani et al., 2008b), and thus can be an ally in the treatment for...
pregnancy-related cholestasis. This protection could occur mainly because as demonstrated by Arola-Arnal et al. (2013), flavonoids and grape seed metabolites are widely distributed in plasma and maternal tissues thus having a beneficial effect.

Although, there is no consensus in the literature as to whether consumption of substances rich in polyphenols is beneficial during pregnancy and to the offspring. A study by Arola-Arnal et al. (2013) demonstrated that the placenta acts as a barrier to flavonoids and their metabolites, verifying that the transport across the placenta was not efficient compromising the trans-generational beneficial effect (Arola-Arnal et al., 2013). In contrast, other studies report that consumption of substances rich in polyphenols with antioxidant and anti-inflammatory activities during pregnancy may interfere with the dynamics of the fetal ductus arteriosus and even constriction of the ductal channel (Zielinski et al., 2010a; Zielinski et al., 2010b). Nonetheless, several other studies, both in vivo and in vitro, have demonstrated the hepatoprotective effect of grape and its derivatives (Buchner et al., 2014; Dani et al., 2008b; Lacerda et al., 2012; Oliboni et al., 2011; Orhan et al., 2007; Rodrigues et al., 2013), thus justifying the search for benefits of juice also in the gestational model.

To this date, no studies in the literature demonstrate the influence of red grape juice in pregnancy. Therefore, the present study aimed the effect of grape juice variety Bordo (Vitis labrusca) on some biochemical and oxidative stress parameters in serum and liver enzymes of pregnant and lactating rats.

MATERIALS AND METHODS

Reagents

Thiobarbituric acid was purchased from Merck (Darmstadt, Germany), and 2,4-dinitrophenylhydrazine (DNPH) was obtained from Sigma (St. Louis, USA). All other reagents were of analytical grade and purchased from local suppliers.

Grape juice

The commercial conventional red grape juice Vitis labrusca variety Bordo was kindly provided by Perini Winery. The juices were from the harvest of 2013 and all from the same lot. The juice used has been previously analyzed and the total acidity (0.70 ± 0.00), volatile acid in acetic acid (0.02 ± 0.00), total sugars (17.4 ± 0.00) relative density (1.07 ± 0.0001), alcohol content (0.3 ± 0.00), pH (3.49 ± 0.01), benzoic acid (0.00 ± 0.00) and sorbic acid levels (0.00 ± 0.00) measured. The juice in question also had its phenolic composition evaluated: total phenolic compounds (5698.67 ± 58.12), resveratrol (0.46 ± 0.00), catechin (3.76 ± 0.16), epicatechin (0.85 ± 0.01), hesperidin (2.03 ± 0.03), rutin (26.44 ± 0.49), gallic acid (0.43 ± 0.09) and chlorogenic acid (24.76 ± 0.32).

Animals

In our study 40 Wistar rats at 12 weeks of age, weighing approximately 200 g from the vivarium of the Methodist University Center IPA were put to mating. The animals had free access to water and a commercial diet containing 20.5% protein (predominantly soybean), 54% carbohydrate, 4% fat, 4.5% fiber, 7% ash and 10% moisture. The animals were kept under a 12h light-dark cycle at a temperature of 22°C±1°C. The pregnancy of rats was confirmed by vaginal swab, verifying the presence or absence of sperm. All experimental procedures were performed with the approval of the Ethics Committee on Animal Use (ECAU) of the Methodist University Center IPA, under protocol number 15/2013.

Treatment

The animals received red grape juice Vitis labrusca for a period of 42 days (21 days gestation + 21 days before weaning). The administration of the grape juice was done every day in two ways: by gavage at a concentration of 7µL/g (Dani et al., 2008b) of body weight or free access. The animals were divided into 4 groups:

- Group 1 (Water): composed of 05 pregnant female Wistar rats at 12 weeks of age, treated with free access to water;
- Group 2 (Water - gavage) consisting of 15 pregnant female Wistar rats of 12 weeks of age that were treated with water at a concentration of 7µL/g of body weight;
- Group 3 (Grape Juice): composed of 05 pregnant female Wistar rats at 12 weeks of age, treated with free access to red grape juice;
- Group 4 (Grape Juice - gavage) consisting of 15 pregnant female Wistar rats at 12 weeks of age, treated with red grape juice at a concentration of 7 µL/g of body weight.

In our study, 40 Wistar rats were put to mate but only 20 rats completed the treatment. For the animals that did not complete the treatment, several causes for this interruption were observed: complication of childbirth (2); seizure (1); uterine prolapse (2); death with unidentified cause (8) and other 7 animals did not become pregnant.

After the offspring breastfeeding, females, previously pregnant, were euthanized by decapitation. Truncal blood was collected and the serum was separated and stored at about 0°C for subsequent analyzes. The liver was also removed, homogenized in KCl 1.5% with manual homogenizer and stored in a freezer (-20°C) until analysis.

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 (Biodin-BS120).

**Oxidative stress parameters evaluated in serum and liver**

The test that evaluates reactive substances to thiobarbituric acid (TBARS) was used to measure the level of lipid peroxidation. These are generated by a heated acidic reaction. This method is considered sensitive to quantify levels of lipid peroxidation, as previously described by Wills (1996). In general, samples were mixed with 10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) and then were heated in boiling water bath for 15 minutes in sealed tubes. 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). The DNPH reacts with protein carboxyls to form hydrazones which can be measured with a spectrophotometer at 370 nm. The results were expressed in nmol/mg protein. The non-enzymatic defenses were determined by sulphydryl 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 absorbance is determined with a spectrophotometer at 412 nm (Arksenov and Markesberry, 2001). The sulphydryl content is inversely correlated to the protein oxidative damage. The results were expressed in nmol/mg protein.

**Determination of antioxidant enzymes in serum and liver**

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 (Bannister and Calabrese, 1987). The results were expressed as U SOD/mg protein. The test for assessing the activity of catalase (CAT) was performed according to the method described by Aebi (1984), which determines the rate of decomposition of 

\[ \text{H}_2\text{O}_2 \] at 240 nm (SP-2200 Spectrophotometer, Bioespectro). The results were expressed as U CAT/mg protein.

**Protein Determination**

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

**Statistical analysis**

Results were expressed as mean and standard error of the mean. *Statistical difference (p<0.05) by two-way ANOVA with Holm-Sidak post-test. HDL: high density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase

**RESULTS**

**Biochemical parameters**

Among the evaluated biochemical markers that included total cholesterol, triglycerides, HDL, ALT, AST, urea and creatinine, it was observed that the grape juice was able to reduce the levels of the enzyme AST (p=0.001) (Table 1). It has been further observed that in animals handled, grape juice was able to reduce the levels of this enzyme (Figure 1C). In other biochemical parameters evaluated in serum.
Figure 1: Urea concentration (mg/dL) (A); Creatinine (mg/dL) (B); AST (U/L) (C); ALT (U/L) (D); total cholesterol (md/dL) (E); HDL (mg/dL) (F) and triglycerides (mg/dL) (G) expressed as mean±SEM in serum from female Wistar rats after lactation treated with red grape juice. *Statistical difference according to ANOVA two-way, followed by post-test Holm-Sidak (p<0.05).

As for the protein damages (Carbonyl) the results show that in the liver as well as in the serum, both factors evaluated (group and handling) were instrumental in changing the levels of protein carbonyls (serum: group p=0.001, handling p=0.017; liver: group p=0.001, handling p=0.001). Showing that the group factor decreased levels of protein oxidation and these levels were increased by the gavage in both biological samples evaluated (Table 2). Thus, in serum, it was possible to observe that the grape juice was able to
reduce protein oxidation as compared to the water group (p=0.001) (Figure 2B). But assessing the handling factor in serum, grape juice did not minimize the protein oxidation caused by the stress of gavage (Figure 2B). However, in the liver, it was verified that the stress generated by gavage was able to increase the levels of protein carbonyls when compared to animals treated with water (p=0.00). When evaluating the group factor, it was noted that grape juice was able to decrease protein oxidation generated by the stress of gavage in the liver (p=0.00) (Figure 2B). No significant differences were observed in the levels of lipid peroxidation and sulfhydryl, both in the liver and in the serum assessed (Figure 2A; Figure 2C).

None of the evaluated factors (consumption or handling) were able to cause change in SOD levels in serum and liver (Table 2). Nevertheless, in the liver, it was found that grape juice increased SOD activity in non-handled animals (p=0.046). Meanwhile the ingestion of grape juice by gavage reduced the activity of this enzyme (p=0.014). (Figure 3A).

In the serum, it was found that both the group factor as the handling factor, were instrumental in changing the levels of CAT (p<0.001; p=0.023) (Table 2), not causing the same effect in the liver (Figure 3B). Also in serum, grape juice was able to reduce the CAT activity in animals unhandled (p=0.001). In contrast, when the factor handling was evaluated, it was possible to notice that red grape juice had the ability to increase the enzymatic activity of CAT in animals that were subjected to the stress of gavage (p=0.001).

No statistical difference was found in the relationship between SOD and CAT enzymes in the liver. Still, in the serum, the handling was the most important factor for the change in the ratio SOD/CAT (p=0.023) (Table 2). When evaluated, the relation between the groups revealed that the stress generated by gavage was able to reduce this ratio in groups treated with grape juice (p=0.011) (Figure 3C).

**DISCUSSION**

In our study, the gestational experimental model with Wistar rats was used to evaluate the influence of chronic treatment with red grape juice *V. labrusca* on biochemical and oxidative stress parameters in serum and liver. Aderimi (2004) showed that biochemical analyzes are extremely important, as they allow the detection of stress conditions, which may be nutritional, environmental or physical. It was observed that the grape juice during pregnancy was able to reduce the levels of AST liver enzyme, not changing the parameters of urea, creatinine, ALT, total cholesterol, HDL and triglycerides.

Urea and creatinine markers were used to evaluate the renal profile, where no statistical differences were observed. Our data corroborate a study conducted with grape seed extract, where there was no reduction of these serum parameters in rats with kidney injury induced by gentamicin (Safa et al., 2010). Meanwhile, in an *in vitro* study, Pinheiro et al. (2010) showed that grape seed extract caused a decrease in serum urea in rats, which had been increased by methotrexate (MTX), a drug used to treat autoimmune diseases (Pinheiro et al., 2010).

The beneficial results of grape juice *V. labrusca* on the AST enzyme found in our experiment are consistent with another study of grape juice, but with a Turkish variety of *V. vinifera*, that showed a reduction on the elevation of the levels of the enzyme in question, caused by CCl4 (Pirinççioğlu et al., 2012). A similar result was found by Orhan et al. (2007), where an alcoholic leaf extract of *V.
Figure 2: Levels of TBARS (nmol/mg) (A); Carbonyl (nmol/mg) (B) and Sulphydryls (nmol/mg) (C) expressed as mean±SEM in serum and liver of female Wistar rats after the lactation period, treated with red grape juice. *Statistical difference according to ANOVA two-way, followed by post-test Holm-Sidak (p<0.05).

*vinifera* in its n-BuOH fraction decreased AST and ALT levels (Orhan et al., 2007). However, there were no significant differences in the levels of ALT liver enzyme. Our data corroborate a study conducted by Lacerda et al. (2014), where the levels of this enzyme were also not affected by organic vine leaf extract of *V. labrusca* in the serum of diabetic mice (Lacerda et al., 2014).

The TC and TG levels in our study have not changed. These findings are in agreement with a research of human serum that made regular consumption of green grape juice (verjuice), typical of Iran region, for 60 days (Zibaeenezhad et al., 2012). In contrast, Hort et al. (2012) observed reduction of TC and TG levels in rats submitted to a hypercholesterolemic diet and treated with a fraction of...
ethyl acetate nonalcoholic (EAF) of a Brazilian wine obtained from *V. labrusca*. Still on lipid profile, the treatment was not able to alter HDL levels, in the meantime, several studies have demonstrated significant increases in this parameter subsequent to the treatment with different types of grape juice (Khadem-Ansari et al., 2010; Castilla et al., 2006; Zibaeenezhad et al., 2012).

As mentioned above, pregnancy is a physiological process, where several metabolic pathways are altered, resulting in a higher O$_2$ consumption and changes in the consumption of energy substrates, with a consequent greater exposure to oxidative stress (Vannucchi et al., 2006). Hormonal changes during pregnancy are constant and thus induce normal physiological changes in the human body. One major

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**Figure 3:** Activity of Superoxide Dismutase (SOD) (USOD/mg) (A); activity of catalase (CAT) (UCAT/mg) (B) and ratio SOD/CAT (C) expressed as means±SEM in serum and liver of female Wistar rats after the lactation period, treated with red grape juice. *Statistical difference according to ANOVA two-way, followed by post-test Holm-Sidak (p<0.05).
change is in the metabolism of lipids, which causes significant changes in the status oxidant/antioxidant (Bukhari et al., 2011; Loy et al., 2013).

This study demonstrated that the treatments were not able to cause differences between groups in the levels of lipid peroxidation in serum and liver of the rats tested. However, a study conducted with diets supplemented with food rich in polyphenols, proved that it was able to reduce the plasma TBARS levels in pregnant ewes (Bubols et al., 2014). Similar results were found in an experiment performed in vitro by Oliboni et al (2011), in liver, kidney and heart, with extract of vine leaves V. labrusca organic and conventional, where thiobarbituric acid was also reduced (Oliboni et al., 2011). In a previous study by the group, organic and conventional grape juices were also able to reduce lipid peroxidation in rat liver subjected to a high-fat diet (Buchner et al., 2014).

Moreover, significant differences were found in carbonyl test, which verifies the oxidation of proteins. In the serum of handled and unhandled animals was possible to observe that the levels of protein carbonyl were reduced by grape juice when compared to the group treated with water. Our data corroborate other two studies of organic and conventional red grape juice, where they were able to reduce protein oxidation in plasma and serum of rats, respectively (Dani et al., 2008b; Rodrigues et al., 2013). In contrast, Bubols et al. (2014) showed an increase in carbonyl protein in plasma from sheep subsequent to a treatment with a preparation of foods rich in polyphenols (Bubols et al., 2014). Note that when in high doses, polyphenols can take a pro-oxidant behavior (Decker, 1997; Raza and John, 2005; Watjen et al., 2005). When assessed protein oxidation in the liver, it was found that red grape juice was able to reduce the levels of this parameter generated by the stress of gavage. Our data are consistent with a study by Rodrigues et al. (2013), also with handled animals, where researchers showed a decrease in the levels of protein carbonyl in rat liver subsequent to treatment with grape juice (Rodrigues et al., 2013). Similar findings were observed in a study of the rat liver and organic and conventional grape juice (Dani et al., 2008b). Importantly, unlike our study, these experiments used some toxic substance to induce oxidative tissue damage. Therefore, the results in this study suggest that grape juice can alter the levels of carbonyl protein in situations where there is damage or not.

The sulphydryl technique is one of the methodologies used to evaluate non-enzymatic defenses. In our study no statistical difference in the sulphydryl levels in serum and liver were observed. However, several studies show that grape derivatives are able to increase non-enzymatic defenses in serum and liver of rats, attributing beneficial effect of these derivatives front of this parameter, noting that these studies use a damage induction model (Buchner et al., 2014; Lacerda et al., 2014; Rodrigues et al., 2013). In gestational model grape juice did not change this parameter.

The human body has some enzymes as first line of defense against oxidative damage, among them are the SOD and CAT (Halliwell, 1994). For the enzymatic activity of SOD, it was found that the treatments were not able to change it in the serum of animals. Although, a study conducted with red wine in mouse plasma showed a decrease in the enzymatic activity of SOD (Gris et al., 2013). This decrease in SOD was also seen in our study, but in the liver and in the handled group. Still on the decrease in SOD activity, Dani et al. (2008b) also showed it in plasma of rats with oxidative damage induced by CCl₄ that were treated with red grape juice (Dani et al., 2008b). Our results also demonstrate that the liver when evaluating factor group on unhandled animals, the grape juice was able to enhance the enzyme activity in question. Our data are consistent with two studies using vine leaf extracts in rat liver, one in vitro and with organic leaf extract of V. labrusca and the other in vivo, conducted with alcoholic leaf extract of V. vinifera (Oliboni et al., 2011; Pari and Suresh, 2008). In the study by Pari and Suresh (2008), the alcoholic leaf extract of V. vinifera was able to restore the SOD levels to levels similar to the control group (Pari and Suresh, 2008).

When observing the data of enzymatic activity of CAT, it was possible to see that in the liver there was no evidence of statistical differences. However, the activity of the enzyme in question in the serum was reduced by grape juice treatment, when evaluating the group factor in animals not manipulated. Similar results were observed in studies of red wine and plasma of mice and red grape juice and liver of rats that received a single dose of PTZ convulsivant (Gris et al., 2013; Rodrigues et al., 2013). In contrast, when evaluated the factor handling, it was found that red grape juice was able to increase the enzymatic activity of CAT in animals that were subjected to the stress of gavage. This increase was also observed in the liver of animals receiving organic grape juice (Dani et al., 2008b) and in plasma from pregnant sheep treated with diets supplemented with a preparation of foods rich in polyphenols (Bubols et al., 2014).

It is known that the ratio SOD/CAT is a parameter that shows the imbalance between antioxidant enzymes SOD and CAT that if recurrent may favor the onset of various diseases (Dal-Pizzol et al., 2001). The results show that the handling was the main factor to change the ratio SOD/CAT in serum. Thus, this ratio was reduced by manipulation in the groups treated with grape juice (Figure 3C). This reduction can also be observed in other two studies with handled animals, where the organic leaf extract of Vitis labrusca and conventional and organic red grape juice were able to reduce the levels of the ratio SOD/CAT on brain cortex and hippocampus of Wistar rats and, striatum and substantia nigra, respectively (Dani et al., 2008a; Wohlenberg et al., 2014). Another study also supports our experiment, showing a decrease of this ratio in the liver of animals treated with conventional grape juice (Dani et al., 2008b). In contrast, Schnorr et al. (2011) showed an increase in SOD/CAT ratio in pregnant rats treated with vitamin A compared with the control group (Schnorr et al., 2011).
From the results obtained, it is possible to see that red grape juice *V. labrusca* showed benefits compared to the biochemical and oxidative stress parameters evaluated. It was also effective mainly on the liver enzyme AST and protein oxidative damage, reducing them. Data present in the literature together with our findings strengthen the protective effect of grape juice front of oxidative damage. However, more studies are needed to fully elucidate the mechanisms involved between biochemical parameters, oxidative stress and stress caused by the handling of animals. Meanwhile, the grape juice can already be considered a safe ally in the diet of pregnant women, because it can protect against liver damage.

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