Purple grape juice consumption during the gestation reduces acetylcholinesterase activity and oxidative stress levels provoked by high-fat diet in hippocampus from adult female rats descendants

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Abstract: The enzyme acetylcholinesterase participates in the end of cholinergic transmission and it has been shown that its activity is increased in some diseases that affect the brain, including Alzheimer disease. The objective of this study was to investigate the effect of purple grape juice consumption with or without high-fat diet in the gestational and lactation period on acetylcholinesterase activity and oxidative stress parameters in the hippocampus of female descendants. During pregnancy and lactation, 40 female Wistar rats received a control diet or a high-fat diet, with half of them receiving grape juice. After lactation, the female descendants received water and control diet in ad libitum until euthanasia on the 120 postnatal day. Hippocampus from were removed for analysis of AChE activity, protein oxidation and lipid peroxidation. It was observed that high-fat diet consumption during the pregnancy increased the AChE activity and the grape juice reduced this activity in descendants. The same was observed in protein oxidation, the descendants from high-fat diet had significantly highest values, and grape juice decreased the levels. We conclude that dietary choices during pregnancy can alter the acetylcholinesterase levels and grape juice is an important alternative to improve this function in adulthood.

Key words: Gestation, grape Juice, high-fat diet, Acetylcholinesterase, descendants.

INTRODUCTION

A few years ago we have been observed the study of the importance of maternal life habits during gestation and lactation, influencing the descendants health (Dencker et al. 2016). In this context, we can highlight dietary factors acting as important modulators in disease prevention (Andrade et al. 2014). According to the literature, resveratrol supplementation during pregnancy generates metabolic benefits for pregnant women and, consequently, a healthier future for their offspring. Besides, a recent in vivo study has shown that maternal resveratrol supplementation may improve cognitive performance (Izquierdo et al. 2019).

It is known that grape juice is a food rich in phenolic compounds, and therefore its consumption as a nutritional source may be recommended (Dani et al. 2007). The ingestion of grape juice has gained prominence in in vivo research for its antioxidant and anti-inflammatory power, acting in favor of health (Buchner et al. 2014, Hilger et al. 2015). Recent studies with rats by our research group have demonstrated the grape juice consumption...
during the pregnancy can promote benefits to the pregnancy and her offsprings, strengthening the hypothesis that this food is a great ally for the mother’ health and her offspring (Gonçalves et al. 2017, Wohlenberg et al. 2015). In addition, Dani et al. (2017), observed that the daily consumption of grape juice helps in the modulation of important markers in the brain tissue acting as a coadjuvant of protection against neurological diseases in an experimental model.

About the neuroprotection by grape juice, one of the tissues more explored was the hippocampus (Dani et al. 2010, 2017). The hippocampus is a cerebral region related to memory, spatial memory and of great importance in the limbic system (region that controls emotions) (Koehl & Abrous 2011).

In this sense, the literature related that grape juice consumption demonstrated neuroprotective effect in rats, mainly by the antioxidant response, protecting against the action of reactive species (Dani et al. 2010, Rodrigues et al. 2012).

However, there are few studies that relate the consumption of polyphenols and the cholinergic system (Izquierdo et al. 2019). The cholinergic system has a relationship with learning, memory and mood (Cummings & Back 1998). Therefore, its imbalance is considered one of the hypotheses for Alzheimer’s disease (AD) and other neurodegenerative diseases (Inestrosa et al. 2005). Recent studies, in experimental models, have reported the reduction of acetylcholinesterase (AChE) levels having a neuroprotective effect on AD (Ahmed et al. 2018, Yang et al. 2018). Research evaluating AChE enzyme activity associated with maternal nutrition in in vivo gestational models is still scarce (Menezes et al. 2017). Menezes et al. (2017) demonstrated that green tea (Camellia sinensis) consumption protected against memory deficits related to maternal deprivation. However, the mechanism is not involved with AChE activity but this effect, probably, seems to be related to tea antioxidant properties. This tea consumption promoted the increase of total antioxidant capacity in the hippocampus of rats with maternal deprivation in experimental model (Menezes et al. 2017).

Therefore, the objective of this study was to evaluate if the consumption of purple grape juice associated or not to the consumption of a high-fat diet in the gestational period and lactation alter levels of acetylcholinesterase enzyme activity and parameters of oxidative stress in adult female offspring hippocampus.

MATERIALS AND METHODS

Animals

In this study, we used 78 female Wistar rats and 39 male Wistar rats at 12 weeks of age, weighing approximately 200g from the Animal Center of IPA, where they were placed in mating. The animals were kept under a light and dark cycle of 12h at a temperature of 22 °C ± 1 °C. Day zero of gestation was confirmed by the presence of spermatozoa in the vaginal smear. All experimental procedures were carried out with the approval of the Animal Ethics Committee (CEUA) from Methodist University Center- IPA, under protocol number 019/2014 and 009/2015.

Grape juice

The purple grape juice used was of the conventional type of the variety Bordô kindly provided by the company Fante Bebidas, RS, Brazil. The juices were from the same crop and same lot. The juice was previously analyzed and total phenolic compounds (77.27 ± 2.10) by Folin-Ciocalteau method. The isolated polyphenols were analyzed by HPLC, the content of resveratrol (0.506 ± 0.01), epicatechin (1.95 ± 0.04), naringin (3.37 ± 0.11), rutin (17.41 ± 0.25) and chlorogenic
acid (12.37 ± 0.12) were quantified. No significant amounts of gallic acid and ferulic acid were found (Gonçalves et al. 2017).

Diet control and high-fat diet
The diet control ration used consisted of 20.5% protein (predominantly soy), 54% carbohydrates, 4% fat, 4.5% fiber, 7% ash and 10% moisture. However, the high-fat diet was purchased by Pragsoluções Biociência (Jaú, São Paulo, Brazil) and its composition was supplied by the manufacturer: Corn starch (14.95%), casein (20.00%), starch dextrinized (10.00%), sucrose (10.00%), lard (31.00%), soybean oil (4.00%), microcrystalline cellulose (5.00%), L-cystine (%), choline bitartrate (0.25%), BHT (0.0010%), mineral mix ain 93G (3.50%) and mix vit (1.00%).

Treatment
After confirmation of pregnancy, pregnant rats (n = 40) were randomly divided into 4 (four) groups for treatment with a total of 42 days (21 days of gestation + 21 days of lactation): control diet (CD), high-fat diet (HFD), grape juice and control diet (GJCD) and grape juice and high-fat diet (GJHFD). The pregnant rats had free access to water, grape juice and ration according to the diet of the defined group. After the breastfeeding period, eight female pups from each group received commercial ration and water until the 120 postnatal period when they were euthanized by decapitation. The hippocampus of these animals were removed, homogenized manually in 1.5% KCl and chilled at -18 °C until the analyzes were performed.

AChE activity assay
The hippocampus was homogenized in ten volumes (1:10; w / v) of 0.1 mM potassium phosphate buffer, pH 7.5 and centrifuged for 10 min at 1000 g. The hydrolysis rates were measured at 0.8 mM acetylcholine concentration in 300 μl assay solution with 30 mM phosphate buffer, pH 7.5, and 1.0 mM 5,5-dithiobis- (2-nitrobenzoic acid) (DTNB) at 25 °C. The homogenized tissue supernatant was added to the reaction mixture and preincubated for 3 min. The hydrolysis was monitored by the formation of the dianthiolate of the DTNB at 412 nm for 2-3 min (30 s intervals) (Ellman et al. 1961), with modifications (Scherer et al. 2010). All samples were run in triplicate.

Parameters oxidative stress
To evaluate the level of lipid peroxidation, we used the test that evaluates thiobarbituric acid reactive substances (TBARS). This test evaluates the lipid peroxidation index and was measured according to Ohkawa et al. (Ohkawa et al. 1979). Briefly, TBARS reacts with the lipid peroxidation products to form a pink colored compound that is measured in a spectrophotometer (532nm). The results were expressed as nmol TBARS / mg protein. Oxidative damage to proteins was measured by determination of the carbonyl groups, based on the reaction with dinitrophenylhydrazine (DNPH), according to Levine et al. (Levine et al. 1990). DNPH reacts with protein carbonyls to form hydrazones that can be measured spectrophotometrically at 370 nm. The results were expressed as nmol / mg protein.

Statistical analysis
Kolmogorov-Smirnov test were used to test the normality of the data. And analysis of data by two-way ANOVA, followed by Tukey’s post-test. Data were expressed as mean ± S.E.M and p <0.05 indicated a significant difference. All analyzes were performed using Sigma Plot software version 11.0 (Systat Software, San Jose, CA, USA).
RESULTS

Regarding AChE enzyme activity, we observed an increase of its values in the HFD diet (1.68 ± 0.15) compared with CD (1.24 ± 0.06), (p <0.05). And the juice reduced AChE activity (1.20 ± 0.06) in relation to the group that did not consume the grape juice in both diets (1.72 ± 0.15) (p <0.05). When evaluated the interaction between the factors (diet and beverage) it was observed that the high-fat diet increased enzyme activity only in the group that did not receive grape juice (2.07 ± 0.28), and the protected grape juice increased (1.29 ± 0.10) (p <0.05). The groups that received grape juice, both in the control and high-fat diet presented the lowest values for Ache activity (p <0.05) (Figure 1).

A similar result was observed at levels of protein oxidation. Evaluating the factors alone, we observed that the group that received the high-fat diet presented the highest values (8.48 ± 1.77) in relation to the control diet group (4.06 ± 0.72), (p <0.05). Regarding the beverage factor, the group that received grape juice presented the lowest values (4.60 ± 0.75) in relation to the group that consumed only water (7.94 ± 0.76), (p <0.05). In the interaction of the factors, we observed that the group that consumed a high-fat diet in association with non-consumption of grape juice (11.11 ± 3.35) had the highest values of protein oxidation, and grape juice prevented this increase (5.86 ± 1.18) (p <0.05) (Figure 2). However, when analyzing lipid peroxidation levels, we observed no significant difference between the factors alone or in the interaction between them (Figure 3).

DISCUSSION

This study is the first to investigate the relation between the consumption of purple grape juice and the association of the intake of a high-fat diet during the gestation and breastfeeding period, evaluating AChE levels and parameters of oxidative damage in their adult offspring.
In our study we can observe that consumption of a diet rich in fat during the gestational period increases AChE enzyme levels, as well as protein damage in daughters after adulthood. The deleterious effect of the high-fat diet has been demonstrated in the study by Gonçalves et al. (2017), in which the descendants of mothers who received a high-fat diet had a reduction in the global levels of histone acetylation in the hippocampus of male offspring in adulthood, is linked to gene silencing related to brain function. Soares et al. (2017), showed that cafeteria diet consumption in mice caused weight gain, body fat accumulation, increased glucose, cholesterol, and triglycerides, associated to recognition memory deficits and increased reactive species (RS) levels and acetylcholinesterase (AChE) activity in the hippocampus. However, white, green, red and black (*Camellia sinensis*) significantly reduced these levels, showing benefits.

Previous studies of our group have already demonstrated the benefits of grape juice consumption in a transgenerational model on biochemical parameters (Hilger et al. 2015). The results showed that the grape juice consumption generated benefits since purple grape juice decreased AChE enzyme activity in the hippocampus about control groups in both diets, proving their protective effect on brain tissue. As there is no literature research relating these factors, we can highlight studies using resveratrol a polyphenol found in grape and its derivatives. Schmatz et al. (2009), investigated the effect of the administration of resveratrol on levels of AChE in brain tissue and serum of diabetic rats. They observed the prevention of increased AChE activity in the groups receiving resveratrol when compared to the control group, demonstrating that this compound may modulate the cholinergic neurotransmission and the counterpoint improve cognition. Besides, recent studies have shown the beneficial

**Figure 2.** Carbonyl levels (nmol / mg protein), expressed as mean ± standard error of the mean in female pups hippocampus of Wistar rats treated with purple grape juice during the gestational period. * Difference between diets in groups that received water. ** Difference between groups in relation to drink factor. # factors Interaction, higher value in the group that received hyperlipidic diet and water. Statistical difference according to two-way ANOVA, followed by Tukey test, p <0.05.
effects of grape derivates. Chen et al. (2017) evaluated the effects of consumption of grape seed polyphenols extract, for thirty days, in rats in a chronic brain hyperperfusion model, in rats, recognized as the primary cause of dementia in Alzheimer’s disease. Also, the grapevine consumption was able to decrease AChE levels in the hippocampus, concluding that the effects of oral administration of grape seed extract have these effects due to its cholinergic system preservation effects. Ma et al. (2018) showed that the Vitis Vinifera flavones (VTF) inhibited AChE activity and increased hippocampal acetylcholine levels in an experimental model of Alzheimer’s disease. They concluded that the VTF improves acetylcholine levels precisely due to AChE inhibition. Considering that the acetylcholine is an essential neurotransmitter of the memory-related cholinergic system and learning, these data demonstrate the importance of polyphenols in this system.

It is known that the population aging in Brazil will increase significantly in the next 20 years, so it is believed that the number of diagnoses of neurodegenerative diseases will increase successively (Instituto Brasileiro de Geografia e Estatística (IBGE) 2016). In this sense, studies that demonstrate the importance of maternal feeding in their descendants in all phases of life become increasingly significant. Therefore, in our study evaluating the interaction of diet and drink factors, it was observed that the high-fat diet had the highest values of AChE and protein oxidation in the groups that did not receive grape juice. This association between AChE and oxidative stress was also observed by Soares et al. (2017), in the study with cafeteria diet and teas. In this study, the author showed that the increase of free radical formation could induce alterations in AChE activity, and the consumption of the tea was able to maintain cholinergic signaling and prevent oxidative stress. However, the study Chen et al. (2017), also evaluated oxidative stress parameters. Unlike our study, the grape seed polyphenol extract was able to decrease lipid peroxidation.

**Figure 3.** TBARS levels (nmol / mg) expressed as mean ± standard error of the mean in female pups hippocampus of Wistar rats treated with purple grape juice during the gestational period. Statistical analysis using two-way ANOVA, followed by Tukey’s after test.
levels in the hippocampus of rats submitted to the chronic brain hyperperfusion model and increase enzyme levels. Antioxidants superoxide dismutase, catalase and glutathione peroxidase, again demonstrating a protective effect against increased free radicals in neurodegenerative diseases.

The main objective of this study was to show the deleterious effects of the hyperlipid diet and the beneficial effect of the consumption of grape juice during gestation to the offspring in an experimental model. This is the first study to demonstrate these effects on AchE activity and oxidative stress damage, showing that maternal choices can impact offspring health at all stages, from adulthood to aging, when AD is more prevalent. Taking into account all the results obtained, it is possible to conclude that grape juice reduced the effects of the hyperlipidic diet. In addition, we verified, after HPLC analysis, that grape juice is an important source of polyphenols, which probably could be related (Mandel & Youdim 2004). Epidemiological data from human and new animals suggest that polyphenols may decrease the incidence of dementia, AD and Parkinson’s disease (Mandel et al. 2008). In a recent study Izquierdo et al. (2019) evaluated the effects of resveratrol supplementation on maternal feed on offspring on epigenetic markers and oxidative stress markers, and the results showed that resveratrol might slow the progression of various neurodegeneration signals as cognitive impairment in the next two generations, stressing the importance of the data found in this study. However, the mechanisms involved in the neuroprotection of grape juice offspring against different types of brain injury are not complete. In this way, additional studies are required to better understand these mechanisms, probably by epigenetic pathways.

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