An Acad Bras Cienc (2021) 93(2): e20191002 DOI 10.1590/0001-3765202120191002
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

1916 • 2021

MICROBIOLOGY

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

ISABEL C.T. PROENÇA, LUCIANA K. GONÇALVES, FELIPE SCHMITZ, ALEXANDRE MELLO, CLAUDIA S. FUNCHAL, ANGELA WYSE & CAROLINE DANI

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 improbe 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, problably, 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-cicaulteau 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 to 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.

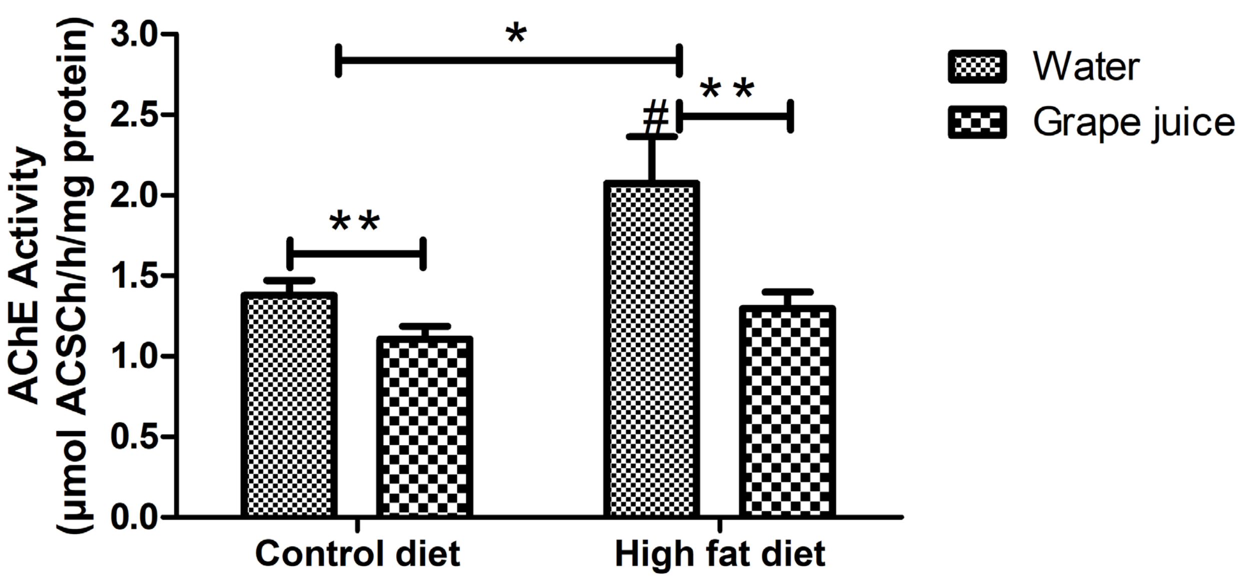

Figure 1. Acetylcholinesterase enzyme activity levels, expressed as mean ± standard error of the mean in the female pups hippocampus of Wistar rats treated with purple grape juice during the gestational period. * Difference between groups in relation to diet. ** Difference in relation to the beverage 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.

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 mices 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

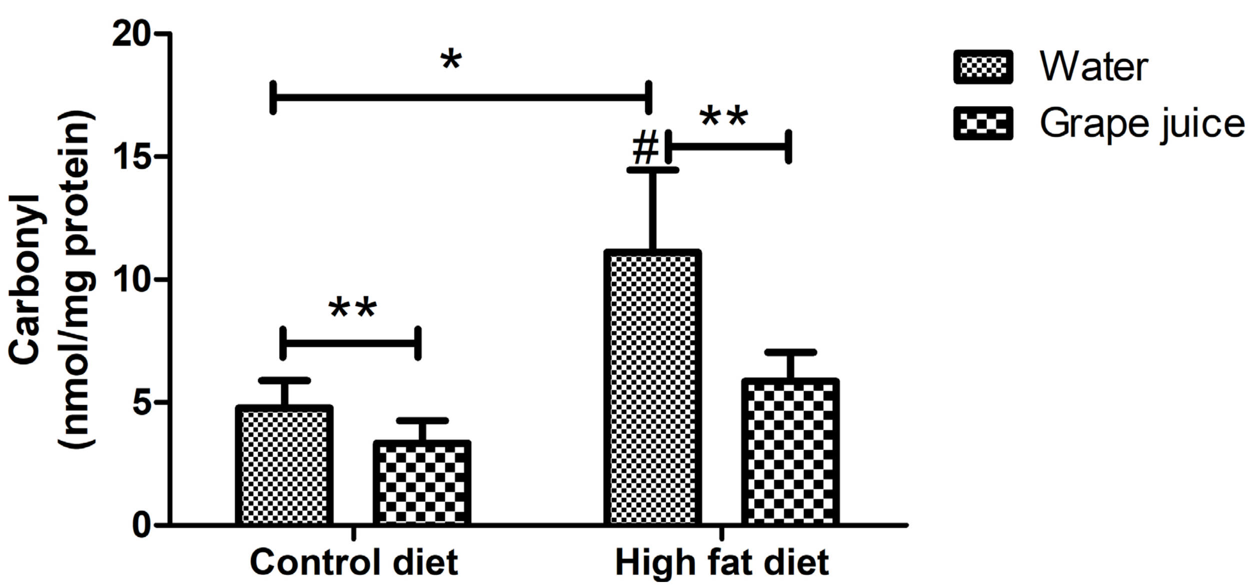

Figure 2. Carbonyl levels (nmol / mg protein), expressed as mean ± standard error of the mean in female pups hippocampus of Wistar rats treated with purple grape juice during the gestational period. * Difference between diets in groups that received water. ** Difference between groups in relation to 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) inhinited 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 inhinition. 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

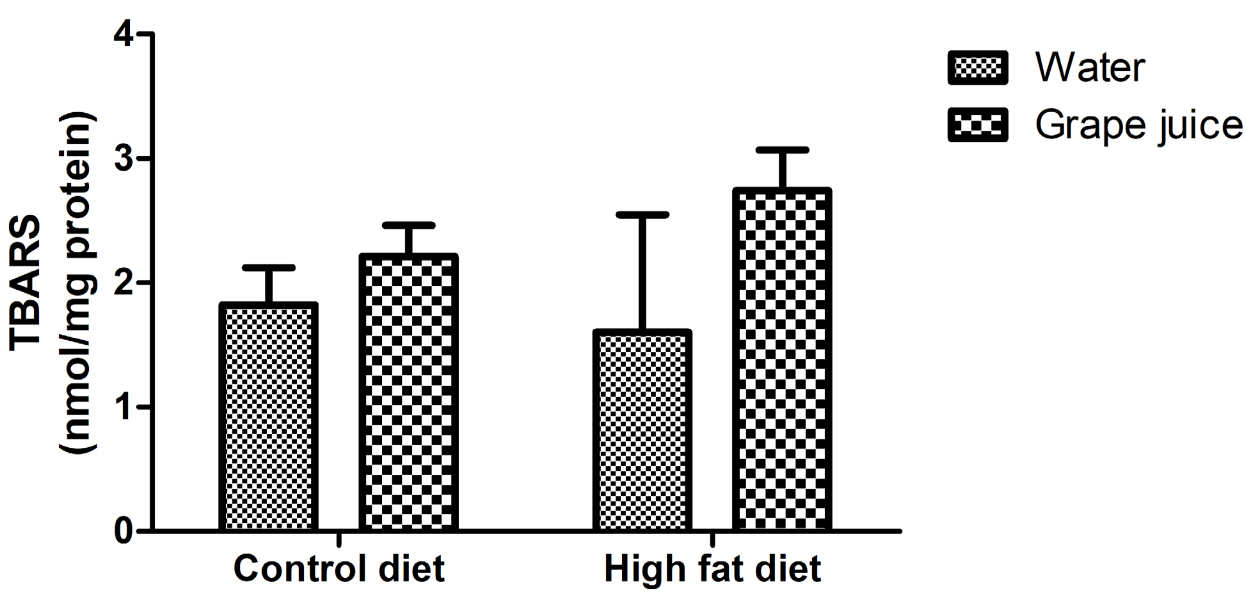

Figure 3. TBARS levels (nmol / mg) expressed as mean ± standard error of the mean in female pups hippocampus of Wistar rats treated with purple grape juice during the gestational period. Statistical analysis using two-way ANOVA, followed by Tukey's after test.

levels in the hippocampus of rats submitted to the chronic brain hyperperfuction 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 migth 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.

Acknowledgments

We thank Helena Biasibetti by technical support. This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS)/Brazil, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq/Brazil, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Instituto Brasileiro do Vinho (IBRAVIN). There is no conflict of interest associated with this study.

REFERENCES

AHMED M, RAZAQ A, RAZAQ A, MUSHTAQ N & ALI KHAN R. 2018. *In vitro* kinetics and inhibition of krait snake ' s venom acetylcholinesterase by Calligonum polygonoides extract in relation to the treatment of Alzheimer 's disease. Iran J Basic Med Sci 21: 869-872.

ANDRADE FO ET AL. 2014. Exposure to lard-based high-fat diet during fetal and lactation periods modifies breast cancer susceptibility in adulthood in rats. J Nutr Biochem 25(6): 613-622.

BUCHNER I ET AL. 2014. Hepatoprotective and Antioxidant Potential of Organic and Conventional Grape Juices in Rats Fed a High-Fat Diet. Antioxidants 3(2): 323-338.

CHEN C, ZHENG Y, WU T, WU C & CHENG X. 2017. Oral administration of grape seed polyphenol extract restores memory deficits in chronic cerebral hypoperfusion rats. Behav Pharmacol 28: 207-213.

CUMMINGS JL & BACK C. 1998. The cholinergic hypothesis of neuropsychiatric symptoms in Alzheimer's disease. Am J Geriatr Psychiatry 6(2): S64-S78.

DANI C, ANDREAZZA AC, GONÇALVES CA, KAPIZINSKI F, HENRIQUES JAP & SALVADOR M. 2017. Grape juice increases the BDNF levels but not alter the S100B levels in hippocampus and frontal cortex from male Wistar Rats. An Acad Bras Cienc 89: 155-161.

DANI C, OLIBONI LS, AGOSTINI F, FUNCHAL C, SERAFINI L, HENRIQUES JAP & SALVADOR M. 2010. Toxicology *in Vitro* Phenolic content of grapevine leaves (Vitis labrusca var . Bordo) and its neuroprotective effect against peroxide damage. Toxicol Vitr 24(1): 148-153.

DANI C, OLIBONI LS, VANDERLINDE R, BONATTO D, SALVADOR M & HENRIQUES JAP. 2007. Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food Chem Toxicol 45(12): 2574-2580.

DENCKER A, PREMBERG Å, OLANDER EK, MCCOURT C, HABY K, DENCKER S, GLANTZ A & BERG M. 2016. Adopting a healthy lifestyle when pregnant and obese - an interview study three years after childbirth. BMC Pregnancy Childbirth 16(1): 1-10.

ELLMAN GL, COURTNEY KD, ANDRES JR V & FEATHERSTONE RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2): 88-95.

GONÇALVES LK, SILVA IRV, CECHINEL LR, FRUSCIANTE MR, MELLO AS, ELSNERA VR, FUNCHAL C & DANI C. 2017. Maternal consumption of high-fat diet and grape juice modulates global histone H4 acetylation levels in offspring hippocampus: A preliminary study. Neurosci Lett 661: 29-31.

HILGER DK ET AL. 2015. Purple Grape Juice, an Important Flavonoids Source, Influence in Biochemical Parameters in Offspring of Wistar Rats. Food Nutr Sci 6: 683-691.

INESTROSA NC, SAGAL JP & COLOMBRES M. 2005. Acetylcholinesterase interaction with Alzheimer amyloid beta. Subcell Biochem 38: 299-317.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. 2016. Síntese de Indicadores Sociais Uma análise das condições de vida da população brasileira.

IZQUIERDO V, PALOMERA-ÁVALOS V, LÓPEZ-RUIZ S, CANUDAS AM, PALLÀS M & GRIÑÁN-FERRÉ C. 2019. Maternal Resveratrol Supplementation Prevents Cognitive Decline in Senescent Mice Offspring. Int J Mol Sci 20: 1134.

KOEHL M & ABROUS DN. 2011. A new chapter in the field of memory: Adult hippocampal neurogenesis. Eur J Neurosci 33(6): 1101-1114.

LEVINE RL, GARLAND D, OLIVER CN, AMICI A, CLIMENT I, LENZ AG, AHN BW, SHALTIEL S & STADTMAN ER. 1990. Determination of Carbonyl Contentin in oxidatively Modified proteins. Methods Enzymol 186(1983): 464-478.

MA L, XIAO H, WEN J, LIU Z, HE Y & YUAN F. 2018. Possible mechanism of Vitis vinifera L. flavones on neurotransmitters, synaptic transmission and related learning and memory in Alzheimer model rats. Lipids Health Dis 17: 152.

MANDEL S & YOUDIM MBH. 2004. Catechin polyphenols: Neurodegeneration and neuroprotection in neurodegenerative diseases. Free Radic Biol Med 37(3): 304-317.

MANDEL SA, AMIT T, WEINREB O, REZNICHENKO L & YOUDIM MBH. 2008. Simultaneous manipulation of multiple brain targets by green tea catechins: A potential neuroprotective strategy for Alzheimer and Parkinson diseases. CNS Neurosci Ther 14(4): 352-365.

MENEZES J, NEVES BH, SOUZA M & MELLO-CARPES PB. 2017. Green tea protects against memory deficits related to maternal deprivation. Physiol Behav 182: 121-127.

OHKAWA H, OHISHI N & YAGI K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2): 351-358.

RODRIGUES AD ET AL. 2012. Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole. Neurochem Int (60(8): 799-805.

SCHERER EBS, CUNHA MJ, MATTÉ C, SCHMITZ F, NETTO CA & WYSE ATS. 2010. Methylphenidate affects memory, brain-derived neurotrophic factor immunocontent and brain acetylcholinesterase activity in the rat. Neurobiol Learn Mem 94(2): 247-253.

SCHMATZ R ET AL. 2009. Resveratrol prevents memory deficits and the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats. Eur J Pharmacol 610(1-3): 42-48.

SOARES MB, RAMALHO JB, IZAGUIRRY AP, PAVIN NF, SPIAZZI CC, SCHIMIDT HL, MELLO-CARPES PB & SANTOS FW. 2017. Comparative effect of Camellia sinensis teas on object recognition test deficit and metabolic changes induced by cafeteria diet. Nutr Neurosci 8305: 1-10.

WOHLENBERG MF ET AL. 2015. Effect of grape juice on some biochemical and oxidative stress parameters in serum and liver enzymes of pregnant and lactating rats. Issues Biol Sci Pharma Res 3: 37-46.

YANG Y, LIANG X, JIN P, LI N, ZHANG Q, YAN W, ZHANG H & SUN J. 2018. Screening and determination for potential acetylcholinesterase inhibitory constituents from ginseng stem-leaf saponins using ultrafiltration (UF)-LC-ESI-MS. Phytochem Anal 1-8.

How to cite

PROENÇA ICT, GONÇALVES LK, SCHMITZ F, MELLO A, FUNCHAL CS, WYSE A & DANI C. 2021. 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. An Acad Bras Cienc 93: e20191002. DOI 10.1590/0001-3765202120191002.

Manuscript received on August 28, 2019;
accepted for publication on January 27, 2020

ISABEL C.T. PROENÇA[1]
https://orcid.org/0000-0002-2560-1975

LUCIANA K. GONÇALVES[1]
https://orcid.org/0000-0002-4361-661X

FELIPE SCHMITZ[2]
https://orcid.org/0000-0002-9746-0286

ALEXANDRE MELLO[1]
https://orcid.org/0000-0002-7311-045X

CLAUDIA S. FUNCHAL[1]
https://orcid.org/0000-0001-5781-0475

ANGELA WYSE[2]
https://orcid.org/0000-0001-8769-1147

CAROLINE DANI[1,3]
https://orcid.org/0000-0003-0046-7148

[1]Programa de Pós-Graduação em Biociências e Reabilitação-Centro Universitário Metodista - IPA, Cel. Joaquim Pedro Salgado, 80, Rio Branco, 90420-060 Porto Alegre, RS, Brazil

[2]Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600, Santa Cecília, 90035-003 Porto Alegre, RS, Brazil

[3]Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500, Centro Histórico, 90050-170 Porto Alegre, RS, Brazil

Correspondence to: **Caroline Dani**
E-mail: carolinedani@yahoo.com.br

Author contributions

Proença ICT: term, conceptualization, methodology, validation, formal analysis, investigation, data curation, writing, visualization prepation, supervision, project administration. Gonçalves LK: term, conceptualization, validation, formal analysis, investigation, supervision, project administration. Schmitz F: methodology and investigation. Mello A: conceptualization, methodology, supervision. Funchal C: validation, methodology, supervision. Wyse A: methodology, visualization prepation, supervision. Dani C: term, conceptualization, methodology, validation, formal analysis, resources, data curation, visualization prepation, supervision, project administration.