Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (2): 39-42
Published online May 8, 2015 (http://www.cellmolbiol.com)
Received on March 23, 2015, Accepted on April 29, 2015.
doi : 10.14715/cmb/2015.61.2.7

Vitis labrusca leaf extract prevents pentylenetetrazol-induced oxidative damage but not seizures in rats

A. S. Coitinho[1]✉, B. M. da Costa[2], M. T. dos Santos[2], B. Fank[2], C. L. Hackmann[3], M. Salvador[4] and C. Dani[2]

[1] Department of Microbiology, Immunology and Parasitology, Universidade Federal do Rio Grande do Sul, RS, Brazil.
[2] Centro Universitário Metodista IPA, Porto Alegre, RS, Brazil
[3] Interdisciplinary Department in Science and Technology, Universidade Federal do Rio Grande do Sul, RS, Brazil.
[4] Institute of Biotechnology, Universidade de Caxias do Sul, Caxias do Sul, RS, Brazil.

Corresponding author: Adriana Simon Coitinho, Department of Microbiology, Immunology and Parasitology, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. Email: adriana.simon@ufrgs.br

Abstract

Epilepsy is a disorder of the central nervous system characterized by recurrent seizures. It is a very common disease in which approximately 30% of patients do not respond favorably to treatment with anticonvulsants. Oxidative stress is associated with neuronal damage arising from epileptic seizures. The present study investigated the possible anticonvulsant and antioxidant effects of a leaf extract of *Vitis labrusca* in an animal model of seizures induced by pentylenetetrazole (PTZ). The animals received injections of *V. labrusca* extract (10, 30 and 100 mg/kg) or vehicle and, 30 minutes later, they received an injection of PTZ, and were then observed for 30 minutes. The latency time and tonic-clonic seizure time were registered. Oxidative damage in lipids and proteins was quantified in the cerebellum, cerebral cortex and hippocampus. It was observed that the leaf extract were capable of reducing lipid peroxidation and protein oxidation caused by PTZ at all doses tested.

Key words: Epilepsy, V*itis labrusca*, antioxidant, pentylenetetrazole.

Introduction

Epilepsy is a common neurological disorder that causes physical, psychological, and social abnormalities in patients. One percent of the world's population has epilepsy and approximately 30% of patients are considered to be pharmacoresistant (1). Plant extracts can be an important source for the development of alternative and complementary treatments for epilepsy. Several plants reputed to possess antiepileptic properties in different cultures have been found to exhibit anticonvulsant activity in different animal models (2, 3, 4, 5).

Some studies in animal models using plant extracts have been conducted and these extracts were shown to be effective in controlling acute seizures induced by pentilenetetrazol (PTZ), which acts by suppressing the inhibitory effects of some neurotransmitters, particularly gamma-aminobutyric acid (GABA), leading to the depolarization of neurons. Studies have shown that the pharmacological effect of PTZ is mediated by interactions with the ion channel of the GABA-A receptor (6, 2, 7, 8).

It has been reported that moderate consumption of grape products (wine and juice) have protective effects against various diseases. These effects may be attributed to the phenolic compounds present in grapes, since it is known that, among fruits, this fruit is an excellent source of these compounds (9, 10).

V. labrusca is the primary species used for the production of wine and juice in South America. However, there have not been many studies investigating the properties of leaves of *V. labrusca*. Thus, this study investigated the possible anticonvulsant and neuroprotective effects of the extract of *V. labrusca* leaves in an animal seizure model.

Materials and methods

Chemicals

2,4-dinitrophenylhydrazine, 5,5'-dithiobis(2-nitrobenzoic acid), thiobarbituric acid and pentylenetetrazole were obtained from Sigma-Aldrich. All other reagents (Merck and Hexapur) and solvents (Nuclear) were of analytical grade.

Extract preparation

Grapevine *V. labrusca* leaves of the Bordo variety from conventional production were collected in November 2007 at the end of blooming, before fructification, in Flores da Cunha, RS, Brazil. The plant was identified by the Herbarium of Universidade de Caxias do Sul (Caxias do Sul, RS, Brazil). The identification number of the voucher specimen was HUCS31065.

Leaves were extracted with 70% ethanol in a closed circuit at 70ºC for 20 h in a Soxhlet extractor. The solvent was evaporated in a water bath for 5 hours and the residues were diluted in water (0.1 g/mL) before each test. The total phenolic content of the grape leaf extract (GLET) was 19.00±0.05 mg gallic acid/mL and the total flavonoid content was 8.95±0.5 µg rutin/mL. The main phenolic compounds were catechin 4.30±0.03 mg/g of raw extract, resveratrol 0.062±0.003 mg/g of raw extract, quercetin 6.74±0.08 mg/g of raw extract, rutin 55.75±0.10 mg/g of raw extract, kaempherol 1.43±0.01 mg/g of raw extract and naringin 0.77±0.012 mg/g of raw extract.

Animals and treatment

Forty male Wistar rats (ten rats in each group) weighing 250–300 g were used. They were maintained at a temperature of 22–24°C, on a 12-h light/12-h dark

cycle, with free access to food and water. The number of animals was determined by a statistical F test-MANOVA (F=3.21, α=0.05, power=90%). The experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW, publication no. (NIH) 85-23, 1985 and were approved by the local ethical committee at Centro Universitário Metodista IPA. The animals were randomly divided into different experimental groups. The control group received saline (0.9% NaCl) and the others received GLET intraperitoneally (ip) at doses of 10, 30 or 100 mg/kg. The doses of GLET were based on previous studies with *Vitis vinifera* leaf extract (11).

PTZ-induced seizures

Immediately after injection of PTZ, the animals were placed individually in plastic boxes and their convulsive behavior was recorded for 30 minutes according to Racine's scale (RS). RS categorizes five stages of intensity, based on the behavioral repertoire of the animals during a seizure, including "mouth and facial movements" (intensity stage 1), "head nodding" (stage 2), "forelimb clonus" (stage 3), tonic-clonic seizures (stage 4) and generalized tonic-clonic seizures characterized by rearing and falling (stage 5) (12). Over a 30-minute observation period, the latency time, tonic-clonic seizures time, total seizures time and stage 5 in the Racine's scale (most intense level of seizure) were registered simultaneously. All effects were reported by two trained observers blinded to the animals' treatment status. After 30 minutes, animals were killed by decapitation. The cerebral cortex, hippocampus and cerebellum samples were collected. Before each assay, livers were homogenized in phosphate buffered saline (pH 7.4) using a ground glass-type Potter-Elvehjem homogenizer and centrifuged for five minutes. The supernatants were used in all assays. All processes were carried out under cold conditions.

Oxidative stress parameters

In this study, lipid and protein oxidative damage were measured. As an index of lipid peroxidation, the formation of thiobarbituric acid-reactive species (TBARS) was used during an acid-heating reaction, as previously described by Wills (13). Results are expressed as nmol of malondialdehyde (MDA)/mg protein. The oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described by Levine *et al.* (14). The results are expressed as nmol/mg of protein. Protein concentrations were measured by the Bradford method (15) using bovine serum albumin as the standard.

Statistical analysis

All values are presented as mean and standard error (S.E.M). All data were subjected to analysis of variance (ANOVA) and Tukey's post-hoc test. Results were considered significant with $p \leq 0.05$. Pearson's correlation was used when necessary. Data were evaluated using a SPSS 18.0 software package (SPSS Inc., Chicago, IL).

Results and Discussion

According to Pan and collaborators (16) and Sreemantula and collaborators (17), some countries make use of the extract of plants of the Vitaceae (grape) family because they have been demonstrated to possess antifungal, anti-stress, hepatoprotective and potential antioxidant effects. Some studies have shown that the intake of *V. labrusca* grape juice enhances antioxidant defense in rats (18, 19).

In Figure 1, it was observed that the administration of different doses of the extract had no significant effect compared to the control group in relation to the latency of seizures induced by PTZ ($p > 0.05$; ANOVA followed by Tukey's post hoc test). It was also observed (Figure 2) that the different doses of the extract had no effect on the duration of tonic-clonic seizures ($p > 0,05$; ANOVA followed by Tukey's post hoc test).

The fact that no anticonvulsant activity was observed in the extract of *V. labrusca* can be explained according to some hypotheses. Firstly, a crude extract of the plant was used. It is known that there are many substances which may have biological activity; however, their positive effects may be masked if these substances are present in small concentrations. Moreover, the dose may have been inadequate to evaluate the parameters investigated, suggesting that different doses should be tested.

Resveratrol, a nonflavonoid polyphenol, has been identified as a potent antiepileptic agent (20, 21) and that an adenosinergic mechanism may play a role in its anticonvulsant activity (20). However, Rodrigues and collaborators (19) observed that although organic and conventional grape juices contain flavan-3-ol derivatives and resveratrol, neither was able to inhibit the sei-

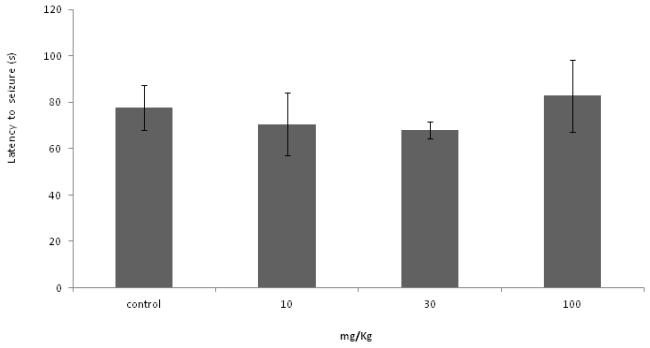

Figure 1. Effect of different doses of *V. labrusca* on the latency of seizures induced by PTZ. Data expressed as mean ± standard error ($p > 0.05$, ANOVA followed by Tukey's post hoc test).

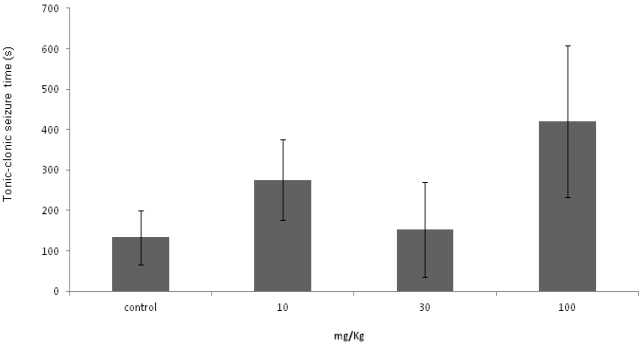

Figure 2. Effect of different doses of *V. labrusca* on tonic-clonic seizure induced by PTZ. Data expressed as mean ± standard error ($p > 0.05$, ANOVA followed by Tukey's post hoc test).

Table 1. Determination of thiobarbituric acid reacting substances (TBARS) (nmol MDA/mg of protein) in the hippocampus, cerebral cortex and cerebellum of rats treated with different concentrations (10, 30 or 100 mg/kg) of GLET in PTZ-induced seizures.

Groups	Hippocampus	Cerebral Cortex	Cerebellum
PTZ (60mg/kg)	20.95±0.61[a]	25.70±0.64[a]	23.06±0.68[a]
GLET (10mg/mL)	8.25±0.89[c]	20.50±0.16[b]	18.15±0.65[b]
GLET (30mg/mL)	9.47±0.78[c]	20,00±2.00[b]	11.70±1.51[c]
GLET (100 mg/mL)	14.00±2.01[b]	14.90±2.34[b]	12.54±1.05[c]

Data are mean ± S.E.M values. Different letters indicate a significant difference according to analysis of variance and Tukey's post hoc test ($p<0.05$) for each parameter evaluated. GLET: grape leaf extract.

Table 2. Determination of carbonyl protein (nmol/mg of protein) in the hippocampus, cerebral cortex and cerebellum of rats treated with different concentrations (10, 30 or 100 mg/Kg) of GLET in PTZ-induced seizures.

Groups	Hippocampus	Cerebral Cortex	Cerebellum
PTZ (60mg/kg)	7.92±0.92[a]	22.03±1.62[a]	9.26±1.01[a]
GLET (10mg/mL)	5.02±0.02[b]	5.86±1.18[c]	1.42±0.28[b]
GLET (30mg/mL)	1.28±0.20[c]	3.87±2.79[c]	3.35±0.63[b]
GLET (100 mg/mL)	3.31±0.54[b]	12.77±0.94[b]	4.11±0.33[b]

Data are mean ± S.E.M values. Different letters indicate a significant difference according to analysis of variance and Tukey's post hoc test ($p<0.05$) for each parameter evaluated. GLET: grape leaf extract.

zures induced by PTZ.

Finally, there is the possibility that the active ingredients of the extract do not have direct action on the GABAergic system; PTZ suppresses inhibitory effects, especially those mediated by GABA, leading to the depolarization of neurons (22, 23). Our results are in agreement with another study which suggest that quercetin and rutin do not affect gabaergic neurotransmission, as they did not change seizures thresholds in PTZ-induced seizures in mice (23). So, the extract may have acted on other receptors.

Some studies have shown that grape products are antioxidant, capable of reducing lipid peroxidation and protein oxidation (19, 24, 25). In this study, we observed a significant decrease in lipid peroxidation and protein oxidation in all groups receiving the leaf extract, when compared with the PTZ group ($p<0.05$) (Table 1). Also shown in Table 1, the highest dose (100 mg/kg) showed higher TBARS levels than the lower doses (30 and 10 mg/kg) only in the hippocampus. In the cerebellum, we observed lower TBARS levels at the higher doses (30 and 100 mg/kg).

This reduction in TBARS caused by the leaf extract was also observed in all tissues in terms of protein oxidation (carbonyl assay) (Table 2). In the hippocampus, we observed that the lowest level was observed at the 30 mg/kg concentration; however, in the cerebral cortex, the lower doses (10 and 30 mg/kg) led to the most significant reduction in protein oxidation. In the cerebellum, all concentrations led to the same important reduction (Table 2).

All results showed a significant reduction in oxidative damage in brain tissues.

Conclusion

In this work, it was observed that *V. labrusca* leaf extract is capable of reducing lipid peroxidation and protein oxidation caused by PTZ. These results show the important benefits of *V. labrusca* leaf extracts, and also indicate that these extracts may lead to the development of new therapeutic strategies for epileptic patients due to their antioxidant effects. It is likely that the antioxidant effect was due to the high concentration of polyphenols (flavonoids) in the plant, since the main content of biologically active compounds consisted of phenolics and ascorbic acid (24, 26). However, no previous studies have been carried on the leaves of *V. labrusca*, the main species grown in South America. Thus, further studies using other seizure models as well as the investigation of isolated fractions of the extract are needed to elucidate the mechanisms of action of *Vitis labrusca.*

References

1. Raza, M., Choudhary, M.I., Medical plants with anticonvulsant activities: Studies in Natural Products Chemistry. Attaur-Rahman: Elsevier. Part C, Bioactive Natural Products. 2000*: 507–553.*

2. Nisar, M., Khan, I., Simjee, S.U., Gilani, H.A., Obaidullah Perveen, H., Anticonvulsant, analgesic and antipyretic activities of *Taxus wallichiana* Zucc, J. *Ethnopharmacol.* 2008, 116: 490–494. doi: 10.1016/j.jep.2007.12.021

3. Rezvani, M.E., Roohbakhsh, A., Mosaddegh, M.H., Esmailidehaj, M., Khaloobagheri, F., Esmaeili, H., Anticonvulsant and depressant effects of aqueous extracts of Carum copticum seeds in male rats. *Epilepsy. Behav.* 2011, 22: 220-225. doi: 10.1016/j.yebeh.2011.07.017

4. Holzmann, I., Cechinel, F.V., Mora, T.C., Caceres, A., Martinez, J.V., Cruz, S.M., de Souza, M.M., Evaluation of behavioral and pharmacological effects of hydroalcoholic extract of valeriana prionophylla standl. from Guatemala. *Evid. Based. Complem. Alternat. Med*. 2011, 1–9. doi: 10.1155/2011/312320.

5. Rathor, N., Arora, T., Manocha, S., Patil, A.N., Mediratta, P.K., Sharma, K.K., Anticonvulsant activity of Aloe vera leaf extract in acute and chronic models of epilepsy in mice. *J. Pharm. Pharmacol*. 2014, 66: 477-85. doi: 10.1111/jphp.12181.

6. Martin, E.D., Pozo, M.A., Animal models for the devel-

opment of new neuropharmacological therapeutics in the status epilepticus. *Curr. Neuropharmacol.* 2006, 4: 33-40. doi: 10.2174/157015906775203002

7. Rogawsky, M.A., Diverse mechanisms of antiepileptic drugs in the development pipeline. *Epilepsy*. 2006, 96:273-294. doi: 10.1016/j.eplepsyres.2006.02.004

8. Schmoll, H., Badan, I., Grecksch, G., Walker, L., Kessler, C., Wagner, A.P., Kindling status in Sprague-Dawley rats induced by pentylenetetrazole. *Am. J. Pathol*. 2003, 162: 1027-1034. doi: 10.1016/S0002-9440(10)63897-7.

9. Dani, C., Oliboni, L.S., Vanderlinde, R., Dias, J.F, Yoneama, M.L., Bonatto, D., Salvador, M., Henriques, J.A.P., Antioxidant activity and phenolic and mineral content of rose grape juice. *J Med Food.* 2009, 12: 188-92. doi: 10.1089/jmf.2008.0090

10. Saha, L., Chakrabarti, A., Understanding the anti-kindling role and its mechanism of Resveratrol in Pentylenetetrazole induced-kindling in a rat model. *Pharmacol. Biochem. Behav*. 2014, 120:57-64. doi: 10.1016/j.pbb.2014.01.010

11. Orhan, N., Aslan, M., Orhan, D.D., Ergun, F., Yeşilada, E., In-vivo assessment of antidiabetic and antioxidant activities of grapevine leaf (*Vitis vinifera*) in diabetic rats. *J. Ethnopharmacol.* 2006, 108:280-286. doi: 10.1016/j.jep.2006.05.010

12. Racine, R., Okujava, V., Chipashvili, S., Modification of seizure activity by electrical stimulation. *Electroencephalogr. Clin. Neurophysiol.* 1972, 32:295–299. doi: 10.1016/0013-4694(72)90178-2

13. Wills, E.D., Mechanisms of lipid peroxide formation in animal tissues, *Biochem. J*. 1966, 3: 667–676.

14. Levine, R.L., Garland, D., Oliver, C.N, Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Stadtman, E.R., Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 1990, 186: 464–478.

15. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976, 72: 248–254. doi: 10.1016/0003-2697(76)90527-3

16. Pan, Z., Agarwal, K.A., Xu, T., Feng, Q., Baerson, S.R., Duke, O.S., Rimando, A.M., Identification of molecular pathways affected by pterostilbene, a natural dimethylether analog of resveratrol. *BMC Med Genomics.* 2008. 1:7**.** doi: 10.1186/1755-8794-1-7.

17. Sreemantula, S., Nammi, S., Kolanukonda, R., Koppula, S., Boini, K.M., Adaptogenic and nootropic activities of aqueous extract of *Vitis vinifera* (grape seed): an experimental study in rat model. *BMC Complement Altern Med.* 2005, 19: 5: 1. doi: 10.1186/1472-6882-5-1

18. Dani, C., Oliboni, L.S, Vanderline, R., Bonatto, D., Salvador, M., Henriques, J.A., Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. *Food. Chem. Toxicol.* 2007, 45: 2574–2580. doi: 10.1016/j.fct.2007.06.022

19. Rodrigues, A.D., Scheffel, T.B., Scola, G., Santos, M.T., Fank, B., de Freitas, S.C., Dani, C., Vanderlinde, R., Henriques, J.A., Coitinho, A.S., Salvador, M., Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole. *Neurochem. Int.* 2012, 60: 799-805. doi: 10.1016/j.neuint.2012.01.009

20. Gupta, Y.K., Chaudhary, G., Srivastava, A.K., Protective effect of resveratrol against pentylenetetrazole-induced seizures and its modulation by an adenosinergic system, *Pharmacology*. 2002, 65(3):170-4 doi: 10.1159/000058044

21. Meng, X.J., Wang, F., Li, C.K., Resveratrol is Neuroprotective and Improves Cognition in Pentylenetetrazole-kindling Model of Epilepsy in Rats. *Indian J Pharm Sci*. 2014, 76(2):125-31.

22. Eloqayli, H., Dahl, C.B., Gotestam, K.G., Unsgard, G., Hadid, I.H., Sonnewald, U., Pentilenotetrazole decrease metabolic glutamate turnover in rat brain. *J. Neurochem.* 2003, 85: 1200-1207. doi: 10.1046/j.1471-4159.2003.01781.x

23. Nieoczym, D., Socała, K., Raszewski, G., Wlaź, P. Effect of quercetin and rutin in some acute seizure models in mice. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014, 54:50-8. doi: 10.1016/j.pnpbp.2014.05.007

24. Dani, C., Oliboni, L.S., Vanderline, R., Bonatto, D., Salvador, M., Henriques, J.A., Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. *Food Chem. Toxicol*. 2007, 45: 2574–2580. doi: 10.1016/j.fct.2007.06.022

25. Scola, G., Scheffel, T., Gambato, G., Freitas, S., Dani, C., Funchal, C., Gomez, R., Coitinho, A., Salvador, M., Flavan-3-ol compounds prevent pentylenetetrazol-inducedoxidative damage in rats without producing mutations and genotoxicity. *Neurosci Lett*. 2013, 534:145-9. doi: 10.1016/j.neulet.2012.11.056

26. Soares, M., Welter, L., KuskoskI, M., Gonzaga, L., Fett, R., Compostos Fenólicos e atividade antioxidante da casca de uvas Niágara e Isabel. Rev. Bras. Frutic. 2008, 30 59-64. doi: 10.1590/S0100-29452008000100013.