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Flavan-3-ol compounds prevent pentylenetetrazol-induced oxidative damage in rats without producing mutations and genotoxicity

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HIGHLIGHTS

- We investigated the protective effects of administering flavan-3-ol extract in rats.
- The extract shows protective effects against neuronal and hepatic damage.
- The extract was shown to be non-genotoxic and non-mutagenic.
- This extract may be used to develop new therapeutic agent against seizures disorder.

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ABSTRACT

Seizure disorder is a chronic condition in the brain that affects approximately 50 million people worldwide. Oxidative stress plays a crucial role in the pathophysiology of this disorder and can cause neuronal injury. Approximately one in three treated patients suffers from seizures regardless of pharmacological intervention, which results in oxidative damage. The present study aims to investigate the possible protective effect of antioxidant-rich *Vitis labrusca* extract on pentylenetetrazol-induced oxidative damage in Wistar rats. Possible behavioral alterations, genotoxic and mutagenic effects of the extract were also evaluated. The results showed that *V. labrusca* extract provides a significant protective effect against oxidative damage to lipids and proteins induced by pentylenetetrazol in the cerebral cortex, cerebellum, hippocampus and liver of rats. Also, the extract did not alter locomotor behavior. Moreover, it was non-genotoxic and non-mutagenic. Our results suggest the possibility of using *V. labrusca* extract as a therapeutic agent to minimize neuronal damage associated with seizures.

1. Introduction

There has been an emerging interest in natural drugs in the past few years. Although several studies have reported that polyphenols improve brain function, their mechanism of action remains poorly understood. In fact, polyphenols were shown to be powerful antioxidants that can prevent reactive species formation by chelating trace elements involved in free radical production, scavenging reactive species, and protecting antioxidant defenses [19].

One of the main causes of cellular damage in the brain is oxidative stress. Reactive oxygen or nitrogen species (ROS or RNS) are directly involved in oxidative damage to proteins, lipids and DNA [19]. Protein oxidative damage can be induced through the reaction between protein and a hydroxyl radical, which introduces carbonyl groups [6]. Moreover, peroxynitrite and nitrous anhydride can cause lipid-peroxidation and DNA single/double-strand breaks that may result in cell death [15].

Excessive production of free radicals is involved in the pathophysiology of seizure disorder [8,10,30,38], where repeated seizures increase the production of ROS and RNS in the brain [8,10,30,38], increasing the likelihood of neuronal death [10,36,48] and subsequent neurodegeneration [38,48]. Furthermore, it has already been shown that seizures can produce oxidative damage in the liver [14]. Despite advances in diagnosis and treatments, the prognosis for patients with seizure disorder remains poor [40], and patients show considerable morbidity. For this reason, it is crucial to research new agents for the management of this disorder.

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Flavan-3-ol compounds [34,37] are polyphenol constituents of *Vitis labrusca*, which present considerable effects [34,35], and modulate cell functionality [12]. However, previous reports only examined a limited number of factors, including in vitro antioxidant and in vivo anti-inflammatory activities [34,35]. Furthermore, these properties raise the possibility that these compounds may be used as therapeutic agents for the prevention of oxidative damage in seizures. For this, the purpose of this study was to expand upon previous findings by investigating the protective effect of the *V. labrusca* winery seeds extract (VLE) against pentylenetetrazol (PTZ)-induced oxidative damage in Wistar rats. To determine possible protective effects of the VLE, oxidative damage and antioxidant defenses were evaluated in the cerebral cortex, cerebellum, hippocampus, and liver of rats. Possible behavioral alterations were also evaluated by the Open Field test. In addition, the possible genotoxic effect of VLE on lymphocytes of rats was analyzed and the mutagenic effect of VLE was evaluated using a recognized model of *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Chemicals

Pentylenetetrazol, thiobarbituric acid, 2,4-dinitrophenylhydrazine, 5,5′-dithiobis(2-nitrobenzoic acid), (−)-epinephrine, guanidine hydrochloride, hydrogen peroxide, and methyl methanesulfonate were purchased from Sigma–Aldrich. All other reagents (Merck and Hexapur) and solvents (Sigma–Aldrich) were of analytical grade.

2.2. Winery wastes seeds extract

Seeds from winery wastes of *V. labrusca* (*cv. Bordo*) were used in this study. The extract was obtained using 5 g of seeds/100 mL of distilled water, under reflux (100 °C; 30 min), filtered through a 0.45 μm pore filter (Millipore) and freeze-dried (Edward freeze dryer) [25]. The extract was solubilized in saline (0.9% NaCl) immediately before use. The major compounds of the extract and detailed methods are listed in Supplementary data.

2.3. Animals, treatments and behavior alterations

Experiments were carried out on 60 male Wistar rats weighing 250–300 g that were allowed free-access to food and water. The experiments were done in accordance with "Guide for the Care and Use of Laboratory Animals, DHEW, publication no. (NIH) 85-23, 1985" and approved by the local ethics committee. Animals were randomly assigned to one of 6 groups (10 animals/group): (1) saline through intraperitoneal (i.p.) injection; (2) 100 mg/kg (i.p.) of VLE; (3) saline plus 60 mg/kg (i.p.) of PTZ; (4) 10 mg/kg (i.p.) of VLE plus 60 mg/kg (i.p.) of PTZ; (5) 50 mg/kg of VLE plus 60 mg/kg (both i.p.) of PTZ; (6) 100 mg/kg of VLE plus 60 mg/kg (both i.p.) of PTZ. The extract or saline was given 30 min before PTZ administration.

Possible locomotor behavior alterations produced by VLE were evaluated using the Open Field test [11]. Anxiety, locomotion and exploratory activities were evaluated in the animals following treatment. The numbers of crossings of square areas of the floor, rearing frequency and fecal bolus were evaluated [21,44]. Measures of behavioral changes were analyzed for each rat 10 min prior to PTZ administration. After PTZ administration, convulsive behavior was analyzed for 30 min according to Racine's Scale [24]. Convulsion parameters and mortality were evaluated. Detailed methods are described in Supplementary data.

2.4. Preparation of tissues and protective effect of VLE

After 30 min of PTZ administration, the animals were euthanized by decapitation and blood and brain were collected. The cerebral cortex, cerebellum, hippocampus, and liver were homogenized. Damage to lipids was monitored by the formation of thiobarbituric acid reactive species (TBARS) [45]. Oxidative damage to proteins was measured by determining carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) [23]. SOD activity was determined by measuring the inhibition of the rate of auto-catalytic adrenochrome formation. One unit was defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50% [5]. CAT activity was determined by the hydrogen peroxide (H_2O_2) decomposition rate. Values were expressed as μmol of H_2O_2 decomposed/min/mg of protein [1]. Determination of protein sulfhydryl content was based on the reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) [2]. Protein concentration was determined by the Bradford method [7]. Detailed methods are described in Supplementary data.

2.5. Genotoxicity assay

Comet assay [9,27] was performed to assess potential genotoxic effects of VLE on lymphocytes of VLE-treated rats and controls. Methyl methanesulfonate (MMS, 8×10^{-5} M) was used as DNA damage positive control. Images of 100 randomly selected cells were analyzed from each sample. The damage was visually scored according to tail size into five classes, from no tail (0) to maximal (4) long tail, resulting in a single DNA damage score for each treatment. Therefore, damage index (DI) ranged from 0 (all cells with no tail, 100 cells × 0) to 400 (all cells with maximally long tails, 100 cells × 4). Detailed methods are described in Supplementary data.

2.6. Mutagenic effect of VLE

Saccharomyces cerevisiae XV185-14c strain was applied to assess potential mutagenic effects of VLE. Cells were grown and incubated in different VLE concentrations (0.05, 0.5 and 5 mg/mL). Survival and induction of mutations for *LYSINE*$^+$, *HISTIDINE*$^+$ or *HOMOSERINE*$^+$ was performed [20,33,43,49]. H_2O_2 (75 mM) was used as a positive control. Detailed methods are described in Supplementary data.

2.7. Statistical analysis

All measurements were performed at least in triplicate. Values were averaged and expressed along with the standard error of mean (SEM), except for the genotoxic and mutagenic assays, which were presented with standard deviation (SD) values. Results were subjected to one-way analysis of variance (ANOVA) and Tukey's post hoc test. To evaluate the significance of the difference between controls and VLE treated rats for the frequency of DNA damage, results were submitted to the independent measures *t*-test ($p < 0.05$) using SPSS 19.0 software.

3. Results

3.1. VLE prevented PTZ-induced mortality

VLE did not induce mortality in the highest concentration assayed (100 mg/kg). Moreover, PTZ caused a progressive increase in seizure intensity, inducing mortality around 40% of PTZ-treated animals. VLE was able to prevent mortality in all concentrations assayed (10, 50 and 100 mg/kg) (Supplementary Fig. 3).