PROTECTOR EFFECT OF BLACKCURRANT (*RIBES NIGRUM* L.) IN SCOPOLAMINE-INDUCED AMNESIA IN MICE

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Introduction

The life expectancy of the world population has gradually increased, resulting in a higher incidence of chronic degenerative diseases. Among the chronic degenerative diseases, Alzheimer's disease (AD) stands out, as it is the most common dementia, accounting for about 70% of the cases in people over 65 years old (WORTMANN, 2012). AD is an age-related multifactorial disease characterized by a range of changes in the anatomy, biology, and function of the brain that causes progressive deterioration of memory and spatial disorientation (BARBOSA et al., 2013).

Studies have shown that individuals with neurodegenerative diseases present changes in cholinergic neurons that lead to memory and learning deficits. The cholinergic system consists of the neurotransmitter ACh, which has a large relevance in the formation of long-term memories and in the retention of memories (PARK et al., 2016). ACh levels are regulated in the synaptic cleft by the action of acetylcholinesterase (AChE; EC3.1.1.7), one of the most important modulatory pathways of the central nervous system (CNS) (SOREQ and SEIDMAN 2001). An increase in the AChE activity may cause decrease of ACh content in the synaptic cleft, affecting cognitive function. (PARK et al., 2016).

Currently, donepezil is used in AD treatment, it is an AChE inhibitor (AChEI), which reduces the ACh hydrolysis and increases the availability of this neurotransmitter in the synaptic cleft (COURTNEY et al., 2004). However, pharmacological treatment is symptomatic and has certain limitations of efficacy, tolerance, and adverse effects, such as cardiovascular and gastrointestinal disorders (FILLIT et al., 2006). Taking into account these information, alternatives that help in the prevention of neurodegenerative diseases are still needed.

Studies have found that antioxidant compounds could have the ability to prevent or ameliorate the effects caused by neurodegenerative diseases. Among these, we can highlight the Cassis (*Ribes nigrum* L), known as Blackcurrant, which is cultivated mainly in temperate Europe, North America, mountainous regions of South America and Asia (BENN et al., 2014). In addition to presenting many health-beneficial properties such as anti-inflammatory, anti-microbial, anti-viral, vasomodulatory, anticarcinogenic, antioxidant and neuroprotective properties (JIA et al., 2012).

These properties are attributed to the fruits, which have high content of ascorbic acid (vitamin C), and, mainly, flavonoids like the anthocyanins cyanidin and delfinidin, which have protective action (VAGIRI et al., 2015).

In this context, since Blackcurrant is rich in biologically active natural compounds, this study proposes to investigate the possible neuroprotective properties of Blackcurrant on the cholinergic system, memory parameters and oxidative stress in a model of memory loss induced by scopolamine in mice.

Materials and methods

In this experiment, 68 Swiss mice (60 days, 25 ± 5 g) were randomly distributed in eight groups, as follows: **Group 1**: Control: saline (n=10); **Group 2**: Blackcurrant 50 mg/kg/saline i.p (n = 10); **Group 3**: Donepezil 5 mg/kg/saline i.p (n=5); **Group 4:** Blackcurrant 50 mg/kg/Donepezil 5 mg/kg/saline i.p (n=5); **Group 5:** Scopolamine 1 mg/kg/oral saline (n = 10); **Group 6:** Blackcurrant 50 mg/kg/Scopolamine 1 mg / kg (n = 10); **Group 7:** Donepezil 5 mg/kg/Scopolamine 1 mg (n = 10); **Group 8:** Blackcurrant 50 mg/kg/Donepezil 5 mg/kg/Scopolamine 1 mg/kg (n = 8).

The animals were treated for 28 days with Blackcurrant (NutriPhy Blackcurrant 100/40805/ Gin: 686831, com 30% de cianidina-3-glicosido) orally once daily at doses of 50 mg/kg. Scopolamine were administered intraperitoneally (i.p.) once daily and at a dose of 1 mg/kg (PARK et al., 2016). The drug donepezil was given orally at a dose of 5 mg/kg once daily. Treatment with scopolamine and Donepezil was initiated after the 7th day of the first administration of Blackcurrant and was last for 21 days. Blackcurrant and/or Donepezil were administered 30 minutes prior to induction by scopolamine.

All compounds were dissolved in saline. In the control group, the animals received vehicle (saline solution) orally and i.p. The solutions were administered in a volume equal to 10 ml/kg orally and 5 ml/kg i.p. On the 22nd day after the beginning of the treatment, the behavioral test of Y maze (TEIXEIRA et al., 2013) was carried to evaluate the spatial memory of the animals. After the 28 days experimental period, the animals were anesthetized and submitted to euthanasia. The cerebral cortex was removed for the determination of AChE and BChE activity (ELLMAN et al. 1961) as well as quantification of reactive species (PEREZ-SEVERIANO et al. 2004). All procedures with animals

were approved by the Ethics Committee on the Use of Animals of the Federal University of Santa Maria under protocol no. 8343230616. Statistical analysis was carried out using two-way ANOVA, followed by Tukey's multiple range test. $P<0.05$ was considered to represent a significant difference in the analyses. All data were expressed as mean ± SEM.

Results and discussion

The Y-Maze test (Fig. 1) was used to analyze the action of the Blackcurrant (BC) on the spatial memory of mice in a scopolamine-induced amnesia model. From the results obtained, it was verified that the number of entries in the arms (Fig. 1A) was significantly higher (72%) and the percentage of alternation (Fig. 1B) was significantly decreased (20%) in the scopolamine group when compared with control group. On the other hand, BC 50 mg/kg treatment reduced the number of entries in the arms and increased the percentage of alternation to the control levels when compared to the Scopolamine group ($p<0.05$). Donepezil 5 mg/kg treatment also had the same effect ($p<0.05$). There were no significant changes in the control groups BC 50 mg/kg, Donepezil 5 mg/kg, BC 50 mg/kg/Donepezil 5 mg kg.

The results show that treatment with scopolamine induced a spatial memory impairment, indicated by the higher number of arms entrances and significantly reduced alternation of animals in the Y-maze test. This is the amnesic effect of this alkaloid, which acts as a competitive antagonist of muscarinic receptors, used to mimic cognitive deficits found in AD in animal models (GHUMATKAR et al., 2015, LI et al., 2016). However, Blackcurrant 50 mg/kg administration for 28 days was able to prevent this memory damage caused by scopolamine. It is known that Blackcurrant has in its composition high content of anthocyanins, mainly cyanidin-3-glycoside, which is attributed potent antioxidant and neuroprotective capacity (CARVALHO et al., 2016). Blackcurrants reduce memory deficit, results that resemble those found in the treatment with the drug Donepezil 5 mg/kg, which is a standard drug used in patients diagnosed with AD. However, this drug causes gastrointestinal effects such as nausea, vomiting and diarrhea (LEE, S. et al., 2014).

In Figure 2, the results demonstrate the effect of BC and Donepezil treatment on AChE (Fig. 2A), BChE (Fig. 2B) activities and in intracellular levels of reactive species (RE) (Fig. 2C) in cerebral cortex mice subjected to scopolamine-induced amnesia. A significant increase in the AChE (34%) and BChE (26%) activity, besides increase in RE levels (55%) was observed in the Scopolamine group when compared to the control group. However, BC 50 mg/kg and/or Donepezil treatment reduced both enzymes activities and reactive species levels in the cerebral cortex when compared to the Scopolamine group ($p<0.05$). In the control groups BC 50 mg/kg, Donepezil 5 mg/kg, BC 50 mg/kg/Donepezil 5 mg/kg no significant changes were observed.

In this study, it was also observed that the administration of scopolamine in the animals increased AChE and BChE activities in the brain. This increase in AChE activity may cause an increased degradation of the ACh neurotransmitter in the brain, which in turn is essential in learning and memory (LI et al., 2016). However, this change was avoided by Blackcurrant 50 mg/kg and Donepezil treatment, selective AChE inhibitor. Thus, through these results, it is suggested that Blackcurrant plays a protective role in the degradation of ACh and may improve memory disorders (GHUMATKAR et al., 2015).

The same way, BChE hydrolysis ACh and others coline esters. However, its mainly function is related with ability of to replace AChE when this enzyme is absent or inhibited. Since the AChE activity has been altered due to the scopolamine-induced amnesia model, BChE also undergoes alteration due to the progression of AD. Studies have suggested that both AChE and BuChE may have roles in the etiology and progression of AD beyond regulation of synaptic ACh levels (MESULAM et al., 2002). This work has corroborated with this date. In addition, the BC 50 mg/kg and/or Donepezil treatment protected the AChE and BChE alterations, contributing to restauration of cognitive and memory deficits observed in the AD patients.

Our results also show that RE levels were significantly increased by scopolamine in the cerebral cortex of animals. Accumulation can promote oxidative damage in important molecules, such as proteins, lipids and DNA, which can result in cellular dysfunctions and mutations. Thus, damage to biomolecules mediated by oxidative stress is an important risk factor that contributes to the progression of neurodegeneration in AD (NUNOMURA et al., 2013).

However, the Blackcurrant was able to reduce the levels of RE, this demonstrate its capacity to act as an antioxidant, since the treatment avoided the deleterious effects of scopolamine. Moreover, the treatment with Donepezil also showed the same effect, being its antioxidant potential against the oxidative damages existing in the AD demonstrated by other authors (GHUMATKAR et al., 2015; LI et al., 2016). According to ATUKEREN et al. (2017) inhibition of AChE by Donepezil improves redox homeostasis in AD, but the mechanisms involved in this restoration of homeostasis following the administration of donepezil are unclear.

Conclusions

Therefore, the results of this work suggest that Blackcurrant administration has antioxidant and neuroprotective properties. Thus, it demonstrates improvement in cognitive deficits in animals submitted to amnesia, which makes this compound a possible therapeutic agent in the prevention of neurodegenerative diseases, such as AD.

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Figures:

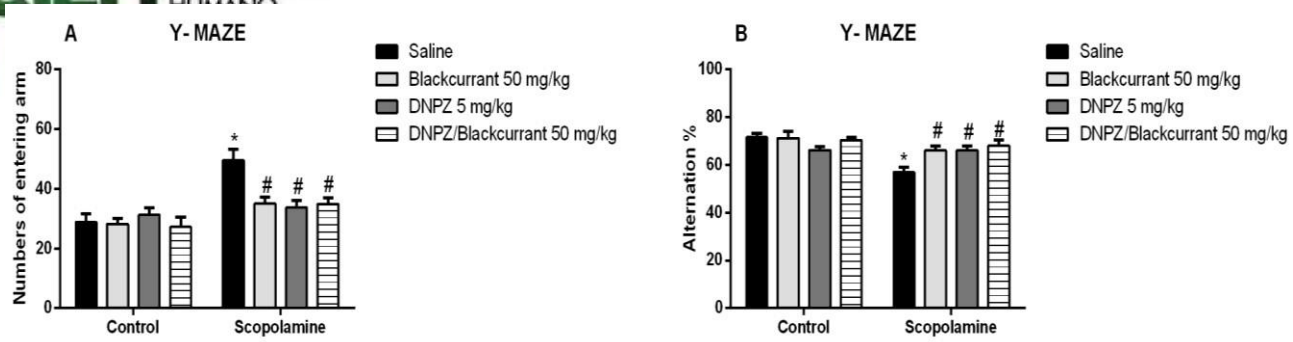

Figure 1: Spatial memory evaluation using the Y-Maze in mice treated with Blackcourrant (50 mg / kg) and Donepezil (5 mg / kg) in a model of amnesia induced by Scopolamine (1 mg / kg). The performance of the mice in this task was measured by the number of their entries in the labyrinth arms (A) and the alternating percentage (B). Statistical analysis was performed using two-way ANOVA, followed by the Tukey test. (*) $p < 0.01$ when compared to the control group. (#) $p < 0.05$ when compared to the scopolamine group. Values are reported as mean ± SEM, (n = 5 -10 per group).

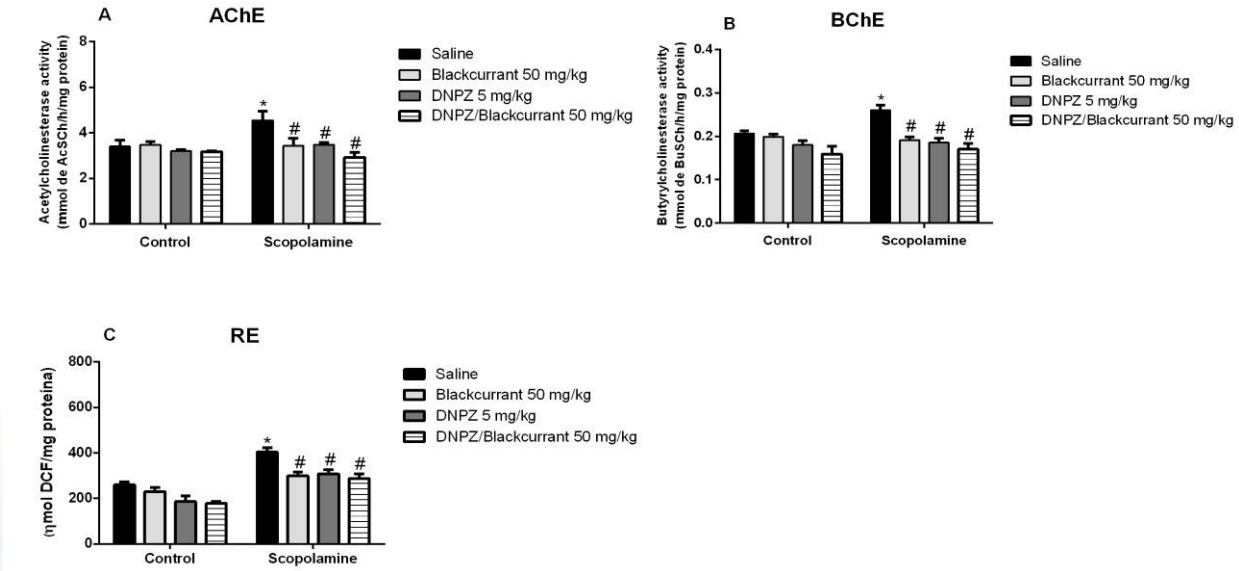

Figure 2: Activities of enzymes Acetylcholinesterase (A) and butyrylcholinesterase (B) and intracellular levels of reactive species (C) in the cerebral cortex of mice treated with BC 50 mg/kg and/or Donepezil 5 mg/kg followed by induction of amnesia by Scopolamine (1 mg/kg). Statistical analysis was performed using two-way ANOVA, followed by the Tukey test. (*) $p < 0.05$ when compared to the control group. (#) $p < 0.05$ when compared to the scopolamine group. Values are reported as mean ± SEM, (n = 5 - 8 per group).