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# Anthocyanins restore behavioral and biochemical changes caused by streptozotocin-induced sporadic dementia of Alzheimer's type

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ABSTRACT

*Aims:* The aim of this study was to analyze if the pre-administration of anthocyanin on memory and anxiety prevented the effects caused by intracerebroventricular streptozotocin (icv-STZ) administration-induced sporadic dementia of Alzheimer's type (SDAT) in rats. Moreover, we evaluated whether the levels of nitrite/nitrate (NOx), $Na^+,K^+$-ATPase, $Ca^{2+}$-ATPase and acethylcholinesterase (AChE) activities in the cerebral cortex (CC) and hippocampus (HC) are altered in this experimental SDAT.
*Main methods:* Male Wistar rats were divided in 4 different groups: control (CTRL), anthocyanin (ANT), streptozotocin (STZ) and streptozotocin + anthocyanin (STZ + ANT). After seven days of treatment with ANT (200 mg/kg; oral), the rats were icv-STZ injected (3 mg/kg), and four days later the behavior parameters were performed and the animals submitted to euthanasia.
*Key findings:* A memory deficit was found in the STZ group, but ANT treatment showed that it prevents this impairment of memory ($P < 0.05$). Our results showed a higher anxiety in the icv-STZ group, but treatment with ANT showed a *per se* effect and prevented the anxiogenic behavior induced by STZ. Our results reveal that the ANT treatment (100 μM) tested displaces the specific binding of [$^3$H] flunitrazepam to the benzodiazepinic site of $GABA_A$ receptors. AChE, $Ca^+$-ATPase activities and NOx levels were found to be increased in HC and CC in the STZ group, which was attenuated by ANT ($P < 0.05$). STZ decreased $Na^+,K^+$-ATPase activity and ANT was able to prevent these effects ($P < 0.05$).
*Significance:* In conclusion, these findings demonstrated that ANT is able to regulate ion pump activity and cholinergic neurotransmission, as well as being able to enhance memory and act as an anxiolytic compound in animals with SDAT.



## Introduction

Anthocyanins (ANTs) belong to the flavonoid family, which present phenolic groups in their chemical structure and give colors to a great variety of flowers and fruits (Table 1) (Veitch and Grayer, 2011; Williams and Grayer, 2004; Yoshida et al., 2009). It has been shown that ANTs are potent antioxidants, and are effective scavengers of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Kahkonen and Heinonen, 2003; Kahkonen et al., 2001), having a clear neuroprotective role (Min et al., 2011). There is evidence that ANTs have beneficial effects on memory and cognition (Shukitt-Hale et al., 2009) improving the memory in old rats and humans (Andres-Lacueva et al., 2005; Krikorian et al., 2010).

Acetylcholinesterase (AChE) is an important regulatory enzyme that rapidly hydrolyzes the neurotransmitter acetylcholine (ACh) released by the cholinergic neurons (Paleari et al., 2008). Several experimental and clinical studies clearly indicate an undisputed major role of ACh in the regulation of cognitive functions (Blokland, 1995). Recently, several therapeutic strategies that enhance AChE activity have been implemented to ameliorate cognitive disorders. Cognitive disorders also affect the generation of membrane potentials and the influx of neuronal $Ca^{2+}$ (Berrocal et al., 2009; Mata et al., 2011).

The $Na^+,K^+$-ATPase and the $Ca^{2+}$-ATPase are key enzymes in the maintenance of electrolyte gradients in excitable cells and neurons (Jimenez et al., 2010; Panayiotidis et al., 2010). The former enzyme is responsible for the active transport of $Na^+$ and $K^+$, and it is necessary

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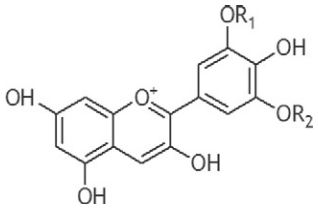
**Table 1**
Structural identification of anthocyanins.




| Anthocyanins | R1 | R2 | Formula | M.W. |
|---|---|---|---|---|
| Cyanidin | OH | H | $C_{15}H_{11}O_6$ | 322.72 |
| Malvidin | $OCH_3$ | H | $C_{16}H_{13}O_6$ | 336.74 |
| Delphinidin | OH | OH | $C_{15}H_{11}O_7$ | 338.72 |
| Petunidin | $OCH_3$ | OH | $C_{16}H_{13}O_7$ | 352.74 |
| Malvidin | $OCH_3$ | $OCH_3$ | $C_{17}H_{15}O_7$ | 366.77 |

to maintain the ionic gradient across membranes and thus it is essential to regulate neuronal excitability (Jimenez et al., 2010; Jorgensen et al., 2003; Kaplan, 2002). The $Ca^{2+}$-ATPase is one of the most powerful modulators of intracellular $Ca^{2+}$ levels (Casteels et al., 1991; Huang et al., 2010; Raeymaekers and Wuytack, 1991). The transient changes in intracellular $Ca^{2+}$ levels regulate a wide variety of cellular processes and cells employ both intracellular and extracellular sources of $Ca^{2+}$ for the activation of signaling pathways and regulation of many physiological and pathological processes (Huang et al., 2010; Missiaen et al., 2000a,b; Ruknudin and Lakatta, 2007).

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, and this disease is characterized by abnormalities in glucose metabolism, reduced glucose utilization and levels of energy rich phosphates (Hoyer, 2004a,b). The intracerebroventricular (icv) injection of STZ in rats has been used as a model of sporadic dementia of AD (Sharma and Gupta, 2001) since it mimics many pathological processes of the disease as impaired brain glucose and energy and leads to progressive deficits in learning and memory (Lannert and Hoyer, 1998).

Considering that AD is the most prevalent neurodegenerative disease worldwide in older adults, we sought to investigate if anthocyanin has the ability to prevent memory deficits induced by icv administration of STZ. We also evaluated the levels of nitrite/nitrate and the activities of enzymes AChE, $Na^+$,$K^+$-ATPase and $Ca^{2+}$-ATPase, which are known to be altered in AD.

## Material and methods

### *Chemicals*

Acetylthiocholine, Trizma Base, acetonitrile, Percoll, Coomassie Brilliant Blue G and streptozotocin (STZ) were purchased from Sigma Chemical Co. (St. Luis, MO, USA). Anthocyanins were extracted and purified from grape skin and are commercially available by Christian Hansen A/S. All other reagents used in the experiments were of analytical grade and of the highest purity.

### *Animals*

Male Wistar rats (3 month year old) weighing 350–400 g were used in the study. They were kept in the Central Animal House of the Federal University of Santa Maria in colony cages at an ambient temperature of 25 ± 2 °C and relative humidity 45–55% with 12 h light/dark cycles. They had free access to standard rodent pelleted diet and water *ad libitum*. All procedures were carried out according to the NIH Guide for Care and Use of Laboratory Animals, and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care. This work was approved by the ethical committee of the Federal University of Santa Maria (23081.003601/2012-63).

### *Administration of drugs to animals*

#### *Intracerebroventricular (icv) injection of streptozotocin*

Adult male Wistar rats (300–350 g) were anesthetized with thiopental (180 mg/kg). The head was placed in position in the stereotaxic apparatus and a midline sagittal incision was made in the scalp. The stereotaxic coordinates for the lateral ventricle (Paxinos and Watson, 1986) were measured accurately as anterio-posterior −0.8 mm, lateral 1.5 mm and dorso-ventral, −4.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Through a skull hole, a 28-gauge Hamilton® syringe of 10 μL attached to a stereotaxic apparatus and piston of the syringe was lowered manually into each lateral ventricle. We used 4 different groups: control (CTRL), anthocyanin (ANT), streptozotocin (STZ), and streptozotocin plus anthocyanin (STZ + ANT). The STZ groups received bilateral icv injection of streptozotocin (3 mg/kg, body weight) which was dissolved in citrate buffer (pH 4.4) (Tiwari et al., 2009). The concentration of STZ in citrate buffer was adjusted so as to deliver 5 μL/injection site of the solution. Rats in the control group received icv injection of the same volume of citrate buffer as in the STZ treated (Scheme 1).

#### *Drug administration*

Seven to ten animals per group were usually tested in the experiments. Rats were treated by gavage with anthocyanin (200 mg/kg body weight) daily per 7 days (around 10 am). The dose of anthocyanin was chosen on the basis of previous studies indicating neuroprotection (Gutierres et al., 2012b; Manach et al., 2004; Saija et al., 1990; Varadinova et al., 2009). The control groups received only vehicle (2 mL/kg gavage of saline, daily per 7 days).

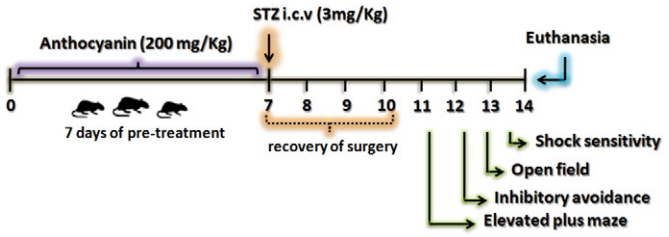
### *Behavioral procedure*

#### *Elevated plus maze task*

At the last day of anthocyanin treatment (7th day), the anxiolytic-like behavior was evaluated using the task of the elevated plus maze as previously described (Frussa-Filho et al., 1999; Rubin et al., 2000a). The apparatus consists of a wooden structure raised 50 cm from the floor. This apparatus is composed of 4 arms of the same size, with two closed-arms (walls 40 cm) and two open-arms. Initially, the animals were placed on the central platform of the maze in front of an open arm. The animal had 5 min to explore the apparatus, and the time spent and the number of entries in the open- and closed-arms were recorded. The apparatus was thoroughly cleaned with 30% ethanol between each session.

#### *Inhibitory avoidance task*

The animals were subjected to training in a step-down inhibitory avoidance apparatus as previously described (Rubin et al., 2000b). After that the animals received icv-STZ (3 mg/kg). Twenty four hours after the training the animals were subjected to test in a step-down inhibitory avoidance task. Briefly, the rats were subjected to a single




**Scheme 1.** Exposure design.