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SHORT COMMUNICATION

Leaf Extract of *Eugenia uniflora* L. Prevents Episodic Memory Impairment Induced by Streptozotocin in Rats

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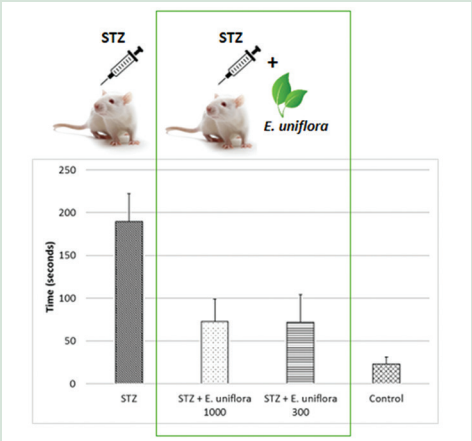
ABSTRACT

Background: *Eugenia uniflora* is a plant found in several countries of South America and is traditionally used in folk medicine as an antimicrobial, anti-inflammatory, and antihypertensive agent. **Objective:** This study aimed to investigate the neuroprotective effect of the ethanolic extract of *E. uniflora* leaves on memory impairment induced by streptozotocin (STZ) in rats. **Materials and Methods:** The ethanolic extract of *E. uniflora* leaves was used to treat Wistar rats which received intracerebroventricular (ICV)-STZ. Animals were allocated to the control group (n = 10), i.e., ICV vehicle solution + ethanol 10%; STZ group (n = 10), i.e., ICV-STZ + ethanol 10%; STZ + *E. uniflora* (300 mg/kg) group (n = 10), i.e., ICV-STZ +300 mg/kg of leaf extract of *E. uniflora*; and STZ + *E. uniflora* (1000 mg/kg) group (n = 10), i.e., ICV-STZ +1000 mg/kg of leaf extract of *E. uniflora*. The animals were submitted to the treatments 24 h after receiving ICV-STZ. The treatments were performed on alternate days over a 30-day period. After this period, the animals were tested in the elevated plus maze to assess learning and memory. **Results:** The STZ group presented a higher transfer latency ($P < 0.001$) which impaired memory regarding the aversive characteristics of the apparatus. The performance in the sessions regarding this parameter of the groups treated with the leaf extract of *E. uniflora* was similar to the control group ($P > 0.05$) for both the doses. **Conclusion:** The leaf extract of *E. uniflora* showed a neuroprotective effect on memory impairment induced by STZ in rats.

Key words: Elevated plus maze, episodic memory, *Eugenia uniflora*, neuroprotection, streptozotocin

SUMMARY

- *Eugenia uniflora* is a typical plant found in South America and has shown several therapeutic properties. Rats received ICV-STZ and were treated orally with the ethanolic extract of *E. uniflora*. Results showed that *E. uniflora* prevented episodic memory impairment in rats exposed to the elevated plus maze, by decreasing the transfer latency parameter.



Analysis of the latency transfer parameter in the Elevated Plus Maze.

Abbreviations Used: STZ: Streptozotocin; *E. uniflora*: *Eugenia uniflora*; ICV: Intracerebroventricular injection; AD: Alzheimer's disease; sAD: Sporadic alzheimer's disease; EPM: Elevated plus maze.

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INTRODUCTION

Sporadic Alzheimer's disease (sAD) is a multifactorial disease with genetic and environmental factors as well as specific metabolic conditions.[1] Some molecular aspects of AD such as impaired glucose metabolism and energy consumption have been widely described and are associated with the progress of the disease,[2,3] as well as with microglia activation, neuroinflammation, and oxidative stress.[4]

Streptozotocin (STZ) is a DNA-alkylating agent which enters the cells exclusively via the glucose transporter 2, leading to death of insulin-producing cells.[5] For this reason, it has been widely used to induce experimental diabetes mellitus in rodents.[6,7] In fact, type 2 diabetes mellitus and AD showed a considerable overlap in relation to pathophysiological mechanisms, such as similar molecular signaling pathways involved in general insulin actions in the body as well as in synaptic neurotransmission, neuronal and glial metabolism, and the neuroinflammatory response in the brain.[8] STZ has been used to investigate several mechanisms associated with sAD. When STZ is administered via intracerebroventricular (ICV) injection in rodents, it disrupts insulin signaling in the brain, leading to insulin resistance,[9] which induces the formation of free radicals. As a result, a progressive neurodegenerative process is initiated, with symptoms similar to those that occur in patients with sAD.[10,11]

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Eugenia uniflora L. is a plant of the family *Myrtaceae* and is widely found in Brazil, where it is popularly known as "Pitanga." It is also found in other countries in South America such as Uruguay, Argentina, and Suriname.[12]

E. uniflora has several therapeutic properties, and its use in folk medicine has been supported by studies showing benefits such as antinociceptive,[13] antioxidant,[12] immunomodulatory,[14] anti-inflammatory,[15] and antihypertensive effects.[16] In addition, some species of *Eugenia* have positive effects on the nervous system such as amelioration of Alzheimer's symptoms in rats submitted to the extracts of *Eugenia jambolana*,[17] neuroprotective properties *in vitro* and *in vivo* using hydroalcoholic extracts of *Eugenia dysenterica*,[18] and anticonvulsant activity against seizures in mice using *Eugenia caryophyllata*.[19]

The aim of this study was to investigate the neuroprotective effect of the ethanolic extract of *E. uniflora* leaves on memory impairment induced by STZ in rats.

MATERIALS AND METHODS

Plant

E. uniflora leaves were collected from a private property (25°25'47.5°S and 49°08'36.6°W) in the municipality of Pinhais in Paraná, Brazil. A voucher specimen was deposited in the Botanical Museum of Curitiba (MBM) with collection number MBM 221042.

Extract preparation

The crude extract was prepared from leaves that were dried and powdered using a Soxhlet extractor. One hundred and seventy milliliters of ethanol was used as extraction solvent for every 20 g of the drug. Subsequently, the extract was concentrated in a rotary evaporator, frozen at −20°C and freeze-dried. The extract was stored in a glass container at room temperature and protected from light. It was diluted in ethanol (10%) and administered orally to the animals on a weekly basis.

Animals

Forty male Wistar rats, 90 days of age, were obtained from the animal facilities at Universidade Positivo. They remained under light/dark cycle of 12 h and temperature of 21°C with water and food *ad libitum*. All the procedures involving animal experimentation were approved by the Ethics Committee on Animal Use in Research of Universidade Positivo under protocol number 337. The experiments were carried out in accordance with ethics recommendations of the Brazilian College of Animals Experimentation and internationally accepted principles for laboratory animal use and care.

Animals were divided into four groups with 10 rats each: control group, in which the animals received vehicle solution ICV and ethanol 10% orally; animals that received STZ-ICV and ethanol 10% orally (STZ group); animals that received STZ-ICV and were treated with 300 mg/kg of leaves extract of *E. uniflora* orally (STZ + *E. uniflora* 300 mg/kg group); and rats that received STZ-ICV and were treated with 1000 mg/kg of the extract orally (STZ + *E. uniflora* 1000 mg/kg group).

Surgery and treatment

Rats were anesthetized with a combination of ketamine (90 mg/kg) and xylazine (12 mg/kg) and were placed in a stereotaxic frame. A sagittal incision in the midline was performed until bregma became clearly visible. According to Paxinos and Watson,[20] the stereotaxic coordinates used for the application of STZ or 0.05 M citrate buffer (pH 4.5) bilaterally into the lateral ventricles from bregma were −0.96 mm in the anteroposterior axis, 1.8 mm in the medial–lateral axis, and 3.6 mm in the dorsal–ventral axis.

Rats of STZ, STZ + *E. uniflora* 300 mg/kg, and STZ + *E. uniflora* 1000 mg/kg groups were subjected to bilateral injections of STZ (3 mg/kg) freshly dissolved in citrate buffer. The same vehicle at the same stereotaxic coordinates was injected into the rats of the control group.

After the surgical procedure, the rats received analgesic treatment for 3 days.

Twenty-four hours after STZ administration, the animals of STZ + *E. uniflora* 300 mg/kg and STZ + *E. uniflora* 1000 mg/kg groups received doses of *E. uniflora* extract orally on alternate days over a period of 30 days. Control and STZ groups received the same number of doses and volumes of 10% ethanol solution orally.

Elevated plus maze

The elevated plus maze (EPM) is made up of four arms (50 cm × 10 cm) at right angles to each other, forming a symmetrical cross in which two arms are closed and the other two are open. In addition, the maze is elevated to a height of 50 cm above floor level.

Episodic memory was evaluated by the test–retest paradigm on the EPM. It consists of repeated exposures to the EPM in a preestablished intertrial interval. Each rat was placed in the open arms facing the center square with free access to all arms for 300 s. Two trials (T1 and T2) were performed with an interval (intertrial interval) of 300 s. Four sessions were performed over 4 consecutive days. Throughout the evaluation period, the experimental groups were randomly tested, and the investigator was blinded to the groups' identities.

Data analysis

Behavioral observations were measured by Pluz MZ v1.1 Software from the time each animal performed the movements. Transfer latency was used as the parameter which indicated 24-h memory retention. All data were analyzed by repeated measures analysis of variance (ANOVA).

All analyses were performed using Statistica 10.0 software (StatSoft, Inc., OK, USA, 2011), and $P \leq 0.05$ was considered to be statistically significant.

RESULTS

The analysis of transfer latency showed a significant difference among the groups ($F_{(3,15)} = 48.20$, $P < 0.0001$). The *post hoc* test demonstrated

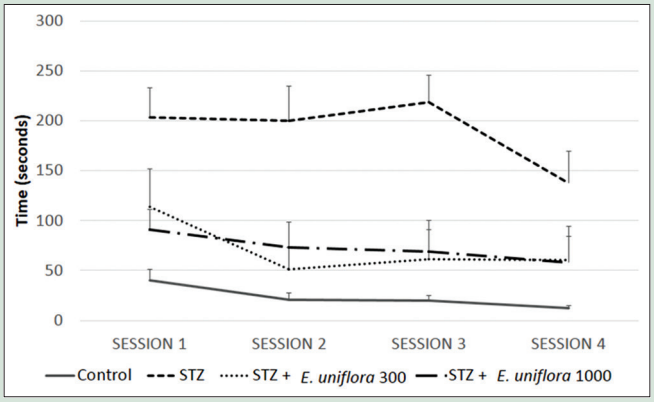

Figure 1: Transfer latency in the elevated plus maze during four sessions. Control group received citrate buffer solution by intracerebroventricular injection and vehicle orally, or streptozotocin intracerebroventricular and vehicle orally (streptozotocin group), or animals that received streptozotocin and were treated with two doses of *Eugenia uniflora* extract (300 mg/kg and 1000 mg/kg orally for 30 alternate days). Group differences were observed ($F_{(3.15)} = 48.20$, $P < 0.0001$)

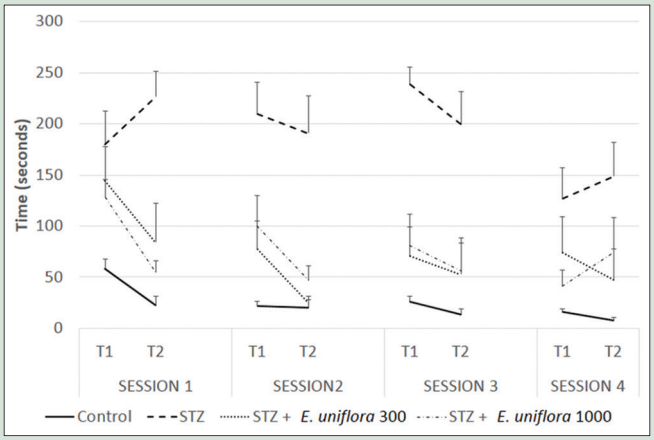

Figure 2: Transfer latency in the elevated plus maze during 4 sessions and within each trial. Control group received citrate buffer solution by intracerebroventricular injection and vehicle orally, or streptozotocin intracerebroventricular and vehicle orally (streptozotocin group), or animals which received streptozotocin and were treated with two doses of *Eugenia uniflora* extract (300 mg/kg and 1000 mg/kg orally for 30 alternated days) during the four sessions and the two trials (T1 and T2) within each session

that the STZ group presented difficulties in consolidating information about the aversive characteristics of the apparatus (Tukey's honestly significant difference, $P < 0.001$), whereas the performance of the groups treated with the extract of *E. uniflora* leaves was similar to the control group ($P > 0.05$), regardless the dose [Figure 1].

In addition, ANOVA showed a significant interaction between Group × Session × Trial factors ($F_{(9,90)} = 2.11$, $P = 0.03$), which indicated that the control group and groups treated with *E. uniflora* extract reduced their latency from the first to the second trial in most sessions, i.e., their performance improved. On the other hand, animals that received STZ-ICV, despite having different latency patterns during the experiment, presented greater latency time in the first and fourth sessions [Figure 2].

DISCUSSION

The ethanolic extract of *E. uniflora* leaves presented a neuroprotective effect on memory impairment in rats that received ICV-STZ, a drug that induces symptoms related to sporadic Alzheimer's disease in rodents, as described in previous studies.[10,11]

The performances of the animals treated with the extract of *E. uniflora* and control animals were similar regarding the consolidation of an aversive context memory formed during previous exposure to the EPM.[21] During the first exposure to the apparatus, the animals explore both the open and the enclosed arms, whereas in the subsequent exposures, the animals tend to spend almost the whole time in the enclosed arms. Thus, time elapsed between the placement of the animal at the end of one of the open arms and its complete entry in one of the enclosed arms is a parameter of acquisition and retention of an aversive context memory called transfer latency.[22,23]

E. uniflora L. is a popular plant in Brazil and in some other countries in South America. Its fruit is edible, and the tea made from its leaves is used in folk medicine as a treatment for digestive problems and hypertension. In addition, several studies have shown its effect as anti-inflammatory, diuretic, antipyretic, and antirheumatic.[24] The compounds found in the leaves of *E. uniflora* include flavonoids, sterols, triterpenes, anthraquinones, and tannins.[24]

The hypothesis presented herein is that the neuroprotective effect observed in the animals treated with *E. uniflora* was the result of its antioxidant and anti-inflammatory properties, which have been described in previous studies.[12,15] Inflammation and oxidative stress are commonly observed in the brains of rats treated with ICV-STZ.[25] Similar results were obtained with the use of the seed extract of *E. jambolana*[17] and leaf extract of *E. dysenterica*,[18] confirming the neuroprotective effect of some species of the *Eugenia* genus. However, the present study is the first report about the neuroprotective effect of the leaf extract of *E. uniflora* species.

CONCLUSION

The results showed a neuroprotective effect of the extract of *E. uniflora* leaves on memory impairment induced by STZ in rats. Therefore, the search for natural compounds that minimize or prevent impairment associated to AD may contribute significantly to the development of new therapeutic strategies in the treatment of neurodegenerative diseases. Further studies are necessary to investigate the mechanism of the neuroprotective effects of *E. uniflora*.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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