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**HEALTH SCIENCES**

# Hippocampal acetylcholinesterase activation induced by streptozotocin in mice is protected by an organotellurium compound without evidence of toxicity

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**Abstract:** The cognitive deficit, which is like Alzheimer's disease and is associated with oxidative damage, may be induced by exposure to streptozotocin. This study aimed to evaluate if the tellurium-containing organocompound, 3j, 5'-arylchalcogeno-3-aminothymidine derivative, interferes with the effects of streptozotocin, as well as to investigate its toxicity in adult mice. Cognitive deficit was induced by two doses of streptozotocin (2.25 mg/kg/day, 48 h interval) intracerebroventricularly. After, the mice were subcutaneously treated with 3j (8.62 mg/kg/day) for 25 days. The effects were assessed by evaluating hippocampal and cortical acetylcholinesterase and behavioral tasks. 3j toxicity was investigated for 10 (0, 21.55, or 43.10 mg/kg/day) and 37 (0, 4.31, or 8.62 mg/kg/day) days by assessing biometric parameters and glucose and urea levels, and alanine aminotransferase activity in blood plasma. 3j exposure did not alter the behavioral alterations induced by streptozotocin exposure. On the other hand, 3j exposure normalized hippocampus acetylcholinesterase activity, which is enhanced by streptozotocin exposure. Toxicity evaluation showed that the administration of 3j for either 10 or 37 days did not cause harmful effects on the biometric and biochemical parameters analyzed. Therefore, 3j does not present any apparent toxicity and reverts acetylcholinesterase activity increase induced by streptozotocin in young adult mice.

**Key words:** Alzheimer's disease, memory deficit, organotellurium compound, streptozotocin, toxicity.

## INTRODUCTION

Alzheimer's disease (AD) affects 30 million people around the world. It is a degenerative disorder characterized by cognitive deficit and compromises short-term memory and, in advanced stages, causes changes in behavior, personality, and judgment (Hung et al. 2016). Etiological speculations point to acetylcholine deficiency, oxidative stress, excessive or insufficient metal levels (Ely 2001, Akinyemi et al. 2015), neurofibrillary plaques, and tangles in the brain (containing an unusual protein and peptide called amyloid) (Hung et al. 2016).

Cognitive deficit like AD may be experimentally induced by intracerebroventricularly (icv)-injected streptozotocin (STZ). This drug causes prolonged metabolic neuronal stress (Nitsch & Hoyer 1991, Lannert & Hoyer 1998) by alterations in insulin receptor (Hoyer et al. 2000). There are long-term and progressive deficits in learning, memory, and cognitive behavior (Lannert et al. 1998, Lannert & Hoyer 1998, Biasibetti et al. 2017).

Rodents icv-exposed to STZ have disorders in cerebral glucose metabolism, oxidative stress, cholinergic deficiencies, neurodegeneration, and cognitive impairment (Costa et al. 2016, 2017, Goyal et al. 2016).

There is an ongoing search for the AD ideal treatment, as the currently available ones are merely palliative and provoke side effects. In this context, several compounds have been investigated, including acetylcholinesterase (AChE) inhibitors (Samadi et al. 2012, Sameem et al. 2017), which are compounds that may inhibit the formation or aggregation of the amyloid peptide (Samadi et al. 2012, Murakami et al. 2016, Samy et al. 2016, Folch et al. 2018), avoid the damage to energy metabolism (Costa et al. 2016), among others.

The synthesis of inorganic and organic compounds containing tellurium, such as tellurols, tellurides, telluranes, and tellurates, is the subject of several scientific investigations (Cunha et al. 2009, Irfan et al. 2020). Immunomodulatory (Cunha et al. 2009), antioxidant (Jacob et al. 2000, Nogueira et al. 2004, Ávila et al. 2008, Borges et al. 2008, Cunha et al. 2009, Rosa et al. 2017), antitumoral (Nogueira et al. 2004, Rosa et al. 2017), antibacterial (Al-Masoudi et al. 2015), anti-inflammatory (Zeni et al. 2001a, b), antifungal (Al-Masoudi et al. 2015), antihelmintic (Cunha et al. 2009), antidepressant (Okoronkwo et al. 2009), and hepatoprotective (Ávila et al. 2011) properties have been reported. Advances in synthesis of these compounds encourage the development of studies of promising agents in therapy of diseases (Nogueira et al. 2004, Irfan et al. 2020).

3j is a new organic compound that contains tellurium (chemical structure, Fig. 1), a 5'-arylchalcogeno-3-aminothymidine derivative. This molecule was synthesized by the Organic Chemistry Laboratory of Universidade Federal de Santa Maria, Brazil. Recently Rosa et al. (2017) observed that 3j has a great antioxidant (*in vitro*) and antiproliferative (evaluated in bladder cancer cell line) potential.

Considering that sporadic AD is associated with oxidative brain injury, this study evaluated whether 3j interferes with the effects caused by STZ to render it as a possible pharmacological agent for the treatment of cognitive Alzheimer type deficits. Besides, the *in vivo* toxicity of this organotellurium was investigated.

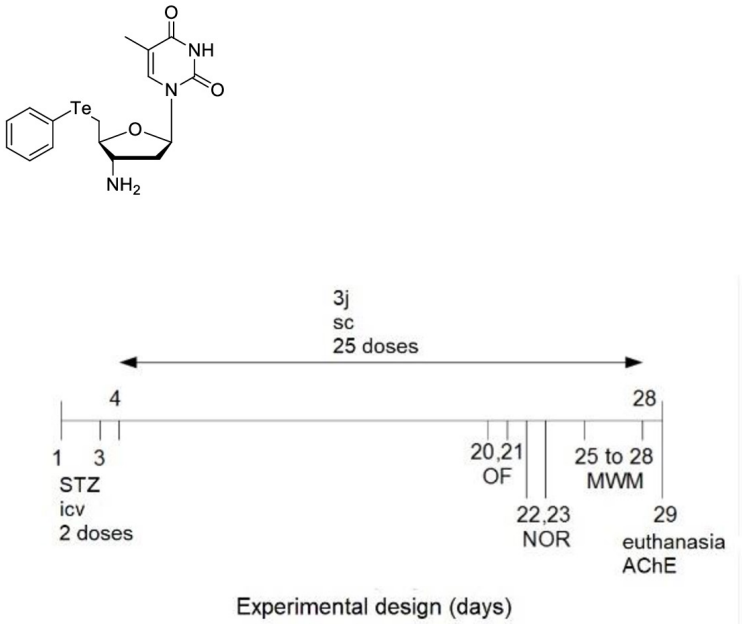


**Figure 1**. **Chemical structure of 3j and treatments and experimental design scheme 3j: organotellurium compound; AChE: acetylcholinesterase; icv: intracerebroventricular; MWM: Morris water maze; NOR: new object recognition; OF: open field; sc: subcutaneous; STZ: streptozotocin.**

## MATERIALS AND METHODS

### Chemicals

Streptozotocin (STZ), acetylthiocholine (ATCh), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical (St. Louis, MO, USA). Urea and alanine aminotransferase (ALT) dosage kits were acquired from Labtest (Lagoa Santa/MG/Brazil). The glucose determination kit was purchased from Bioclin (Belo Horizonte/MG/Brazil). All other chemicals were obtained in analytical grade or from standard commercial suppliers.

### *In vivo* experiments

#### *Experimental animals*

Fifty-five male Swiss mice (young adult, two-month-old, weighing 27±2 g) from the General Animal House of Universidade Federal de Santa Maria were used as test animals. The animals were transferred to the colony room and maintained in opaque plastic cages (16 cm deep x 26 cm wide x 47 cm long) at room temperature of 23 ± 2°C, humidity of 65 ± 5% and with tap water and rodent chow *ad libitum* under a 12:12 h light/dark cycle with the lights on at 07:00 a.m. Each cage contained three or four mice. All manipulations were carried out between 09:00 a.m. and 04:00 p.m. Experiment protocols followed the Ethics Committee on Care and Use of Experimental Animal Resources of Universidade Federal de Santa Maria (Process number 3328120117/2017). All efforts were made to minimize animal suffering and reduce the number of animals used in the experiment.

#### *Cognitive deficit induction*

Animals were divided into four groups (7 or 8 animals *per* group). The first treatment (experimental design, Fig. 1) consisted of icv administration (freehand) of either 20 µL saline (groups I and III) or STZ (groups II and IV) on day 1 (first administration, left cerebral hemisphere) and 20 µL on day 3 (second administration, right cerebral hemisphere) (total volume: 40 µL). STZ was dissolved in saline solution and injected in doses of 2.25 mg/kg/day (Costa et al. 2016, 2017). The icv administration of saline solution or STZ was performed by protocol with the bregma fissure as a reference point based on a previous method (Haley & McCormick 1957, Laursen & Belknap 1986). The animals were anesthetized by isoflurane inhalation and grasped firmly by the loose skin behind the head. The skin was pulled tight and a hypodermic needle (gauge 13 x 0.33 mm) attached to a 1 mL microsyringe inserted perpendicularly through the skull and into the brain. The injection site was 2 mm to either side of the midline on a line drawn through the anterior base of the ears. The insertion depth of the needle was approximately 3 mm. To assess if the solution was administered accurately into the cerebral ventricle, some mice injected with methylene blue solution were euthanized by cervical dislocation, and their brains dissected and macroscopically examined.

#### *3j treatment*

For 3j treatment (day 4 to 28), twenty-five subcutaneous injections (sc) were administrated (one/day in a volume of 2 mL/kg of body weight). The animals from groups I and II received 50% propylene glycol in distilled water. The mice from groups III and IV received 8.62 mg/kg/day of 3j dissolved in 50% propylene glycol solution. The dose was based on the study of Rosa et al. (2017). The sc administration of propylene glycol solution or 3j, which was prepared every other day, was performed with a hypodermic needle (gauge 13 x 0.45 mm) attached to a 1 mL microsyringe inserted into the back of the animals. 3j or propylene glycol was administered after behavioral tests, and body weight was monitored for dosage adjustment.

Groups:

I: sham (2 doses saline + 25 doses propylene glycol)

II: STZ (2 doses STZ in saline + 25 doses propylene glycol)

III: 3j (2 doses saline + 25 doses 3j in propylene glycol)

IV: STZ + 3j (2 doses STZ in saline + 25 doses 3j in propylene glycol)

## Behavioral tests

### *Open field test*

Spontaneous locomotor activity was measured in the open field (OF) test (Walsh & Cummins 1976) on days 17 and 18 of treatment with 3j or vehicle (20 and 21 protocol days: training and test sessions, respectively). This test was performed in the homeroom in a 38 x 38 cm arena with a depth of 30 cm. The floor of the OF was divided into twenty-five equal areas. Each animal was individually placed in one corner of the arena with its nose facing the wall and the number of segments crossed (four-paw criterion) and rearings were recorded in a 5-min session. The arena was cleaned with 40% ethanol before each mouse performed the test.

### *New object recognition test*

The new object recognition task (NOR) was performed according to Bartolini et al. (1996) with some modifications, on days 19 and 20 of treatment with 3j or vehicle (22 and 23 protocol days). This test was performed in the homeroom and in the same arena used for the OF test. The animals were submitted to two identical sessions (day 1 and day 2) that consisted of placing the mouse with its nose facing the wall, equidistant to two objects, for 5 min. On day 1, two identical dark blue octagons called object 1 and 2 (5 cm of height x 6 cm of lower base x 7 cm of upper base) were placed in the arena. On day 2, one of the objects (dark blue octagon) was substituted by a new object called object 3, which was a red star (3 cm of height x 10 cm of diameter). All objects were made of plastic and heavy enough for the mice to be unable to move. The objects and arena were cleaned with 40% ethanol before each mouse and session. Object exploration was defined as the mice sniffing or touching the object with their nose and/or forepaws. The time of exploration of each object was recorded.

### *Morris water maze test*

Spatial learning and memory were assessed using the Morris water maze (MWM) task according to the Morris method (Morris 1984). The maze consisted of a basin (54 cm of depth x 100 cm of diameter) made of navy blue plastic and filled with water (26 ± 1°C) at a height of 41 cm. The mice were transferred to another room before each experiment and allowed to acclimate to the new environment for approximately 1 h before testing. The pool had four out of the water visual cues: white board in different geometric formats containing the red numbers 1, 2, 3, or 4, placed equidistantly on the inner wall of the pool near the edge in quadrants north (N), south (S), east (E) and west (W). An escape platform with 7.5 cm of diameter was hidden 0.5 cm below water level in the middle of the W quadrant. For three consecutive days (acquisition phase), which were days 22 to 24 of treatment with 3j or vehicle (25 to 27 protocol days), the animals were submitted to four trials each day (starting in the W quadrant, then S, then E, and finally N). All mice began the sessions facing the pool wall. Once the mice succeeded in finding the platform, they were allowed to remain at least 40 s after each trial. Whenever the animal failed to reach the escape platform within 1 min (cutoff period), it was retrieved from the water and placed on the platform for 40 s. The time spent in each

quadrant and latencies to reach the platform were calculated as the mean of total time spent in four trials of each day. Twenty-four hours after the acquisition phase (day 25 of treatment with 3j or vehicle, protocol day 28), a probe trial was conducted by removing the platform and placing the mouse next to it and facing the E side (opposite to platform side). The time spent in each quadrant, number of crossings over the former platform site, and time spent to reach the platform site for the first time were recorded for a single 1-min trial.

### *Ex vivo* experiments

Mice were anesthetized by halothane inhalation and euthanized by cardiac puncture for blood collection and tissue dissection on protocol day 29 (24 h after the MWM behavioral test).

The cerebral tissue was removed, and the hippocampus and cortex structures dissected under board-certified personal guidance. The cerebral hippocampi and cortices were homogenized in 100 mM potassium phosphate buffer (KPB), pH 7.1 containing 1% Triton X-100, in a 1:10 weight/volume ratio. The homogenates were centrifuged at 1,200 *g* for 15 min at 4°C to yield the low-speed supernatant (S1) fraction. These samples were stored in a freezer and posteriorly used in the enzymatic assay.

### *AChE specific enzymatic activity*

Enzymatic activity was measured according to the method of Ellman et al. (1961) modified as described in Pereira et al. (2004) in a medium containing 100 mM KPB pH 7.1 and 0.3 mM DTNB. Samples of the hippocampus and cortex S1 fractions were pre-incubated at 25°C for 2 min. The enzymatic reaction was started by adding 0.8 mM of ATCh as substrate and spectrophotometrically monitored at 412 nm for 2 min. Results were expressed in µmol of ATCh/h/mg of protein using the molar absorption coefficient of 13,600. Total protein concentration was measured by the Bradford method (Bradford 1976) using bovine serum albumin as standard.

### *Toxicological evaluation*

Twenty-six young adult mice were used to evaluate the toxicity of the organotelluric compound, 3j. Two treatment doses sc of 21.55 or 43.10 mg/kg/day were administered for 10 days, and two treatment doses of 4.31 or 8.62 mg/kg/day were administered for 37 days. The animals (without fasting, 2 hours after end of dark period) were anesthetized with isoflurane and submitted to euthanasia under board-certified personal guidance 24 h after the last dose of 3j. Blood was collected by cardiac puncture with one 1 mL microsyringe containing heparin as an anticoagulant agent. Plasma was separated by centrifugation. Biometric parameters such as body weight gain, liver, kidneys, and cerebrum weight, and body/organ weight ratio were determined. Glucose and urea levels and ALT enzymatic activity were measured in blood plasma. These biochemistry parameters were chosen as general metabolic assessment and as renal and hepatic function screening.

Glucose was quantified by measuring the product formed with glucose oxidase. Urea was determined by measuring the product formed (indophenol blue) with urease. The Reitman and Frankel method (Reitman & Frankel 1957) was used for determination of ALT enzymatic activity.

### *Data presentation and statistical analysis*

Results are presented as the mean ± standard error. Statistical analysis was performed using one-way or two-way ANOVA followed by Duncan's *post-hoc* test when appropriate. Paired analysis was assessed by the t test. Effects were considered significant when $p < 0.05$.

## RESULTS

### Body weight

Two-way ANOVA (treatment x initial and final weight) revealed the absence of treatment effects. Mice presented similar growth in the course of days [F(1,25) = 533.34; p < 0.001] (Data not shown).

### OF test

Crossing and rearing responses are shown in Fig. 2a and 2b. Two-way ANOVA (4 treatments x 2 sessions) revealed the absence of treatment effects, but significant effect of session [crossing: F(1,25) = 52.86, p < 0.001; rearing: F(1,25) = 20.03, p < 0.001]. In fact, for crossing responses, all groups presented significant differences between training and test sessions, [Paired t test: Sham: $t_{(6)}$ = 3.033 (p = 0.0230); STZ: $t_{(7)}$ = 4.723 (p = 0.0022); 3j: $t_{(6)}$ = 3.206 (p = 0.0185); and STZ+3j: $t_{(6)}$ = 3.768 (p = 0.0093)]. For rearing responses, the Sham and STZ groups presented differences between sessions [Paired t test: $t_{(6)}$ = 3.973 (p = 0.0073) and $t_{(7)}$ = 3.145 (p = 0.0163), respectively].

### NOR test

Two-way ANOVA (treatment x object exploration percentage) showed that the treatment did not affect the training session. On the other hand, the treatment affected object exploration in the test session, as exploration percentage was different [F(1,25) = 6.64, p < 0.05].

The mice equally explored both identical objects in the first NOR session (training – Fig. 3a). In the second session (test – Fig. 3b), which was 24 h after the training, all groups spent more time with the new object than the old one. However, the difference was only significant in the sham group [Paired t test: $t_{(6)}$ = 2.734 (p = 0.034)].

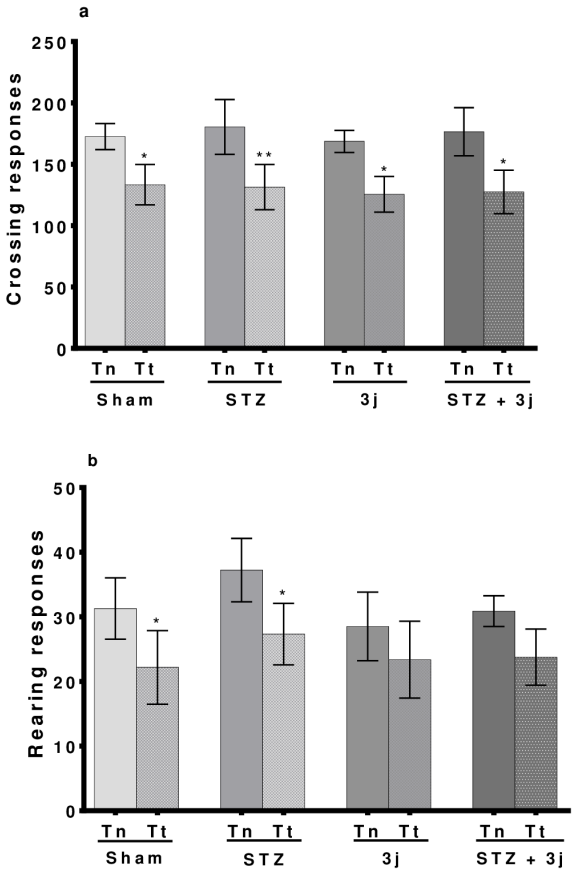


**Figure 2. Crossing (a) and rearing (b) responses in an open field of mice treated icv with vehicle or STZ (2.25 mg/kg/day) for 2 days (days 1 and 3) and sc with vehicle or 3j (8.62 mg/kg/day) for 16 and 17 days, training (Tn) and test session (Tt), respectively. The results are shown as mean ± standard error (n = 7-8). Paired t test: * p < 0.05 and ** p < 0.005.**

### MWM test

One-way ANOVA showed treatment effect on latency in the acquisition phase for days 2 [F(3,25) = 3.271, p < 0.05] and 3 [F(3,25) = 2.848, p < 0.05]. On day 2, latency was higher in the STZ-3j group than in the other groups (Duncan's *post hoc* test: p < 0.05). On day 3, STZ-3j group latency was higher than the sham group latency (Duncan's *post hoc* test: p < 0.05) (Fig. 4a).

In the test session, statistical analyses revealed significant treatment effect on the latency to reach the refuge platform [one-way ANOVA: F(3,25) = 4.368, p < 0.05]. In fact, STZ+3j

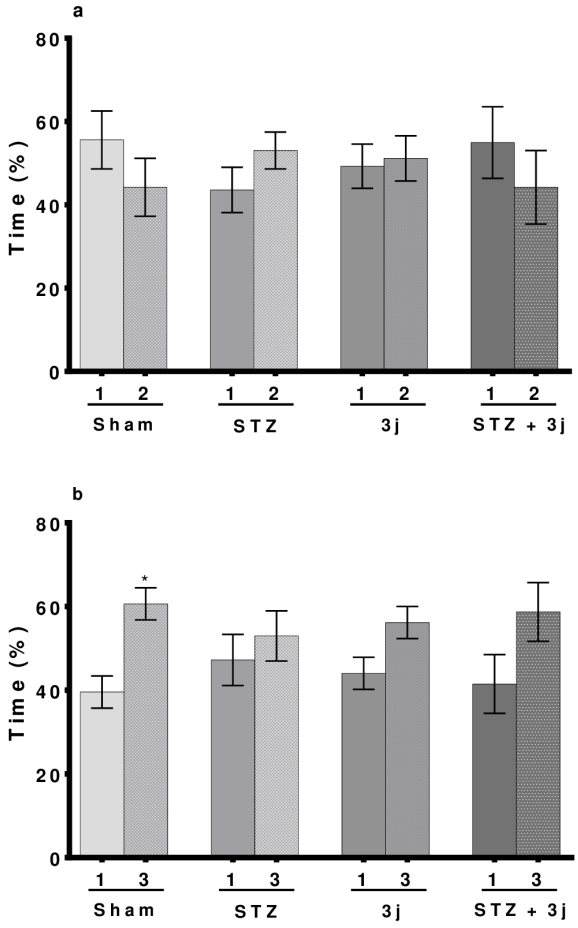


**Figure 3.** **Exploration percentage in relation to total exploration time for both objects in an open field of mice treated icv with vehicle or STZ (2.25 mg/kg/day) for 2 days (days 1 and 3) and sc with vehicle or 3j (8.62 mg/kg/day) for 18 and 19 days, a [1 and 2 (equal objects)] and b [1 (familiar object) and 3 (novel object)], respectively. The results are shown as mean ± standard error (n = 7-8). Paired t test: * p < 0.05.**

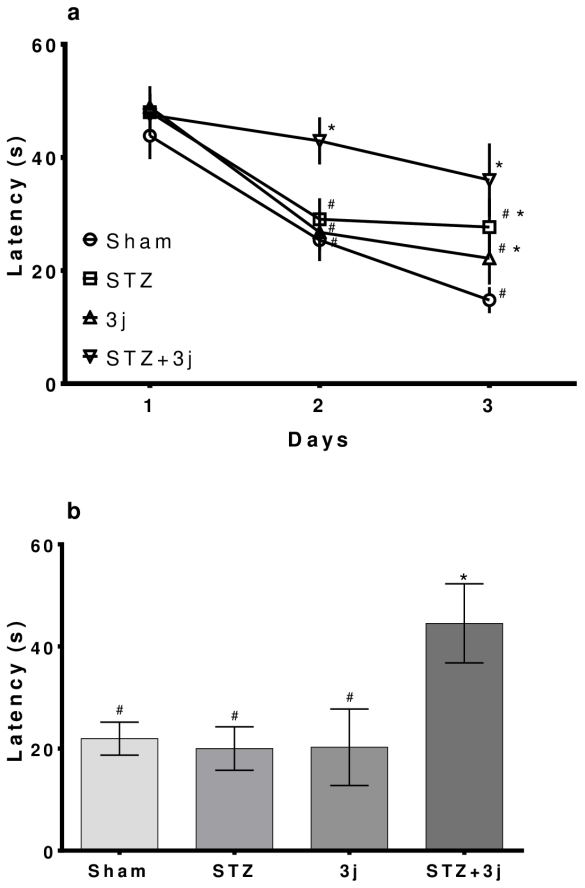


**Figure 4.** **Latency time to reach the refuge platform on days 1, 2, and 3 (acquisition phase) (a) and on test sessions (b) in a Morris water maze of mice treated icv with vehicle or STZ (2.25 mg/kg/day) for 2 days (days 1 and 3) and sc with vehicle or 3j (8.62 mg/kg/day) for 24 days. The results are shown as mean ± standard error (n = 7-8). Duncan's test: different symbols confer significant difference among groups in the same day (p < 0.05).**

presented longer latency to access the refuge than the other groups (Duncan's *post hoc* test: p < 0.05) (Fig. 4b).

## AChE specific enzymatic activity

Hippocampal AChE activity was altered by treatments [One-way ANOVA F(3,25) = 2.961 (p = 0.05)] (Fig. 5a). The STZ increased activity and 3j reversed this alteration [Duncan's *post hoc* test (p < 0.05)]. The AChE activity from the cerebral cortex was not altered by treatments (Fig. 5b).

## *Toxicological evaluation*

Exposure to 3j, sc, during 10 (dose of 21.55 or 43.10 mg/kg/day) or 37 days (dose of 4.31 or 8.62 mg/kg/day) did not alter biometric (body weight gain, cerebral, renal and hepatic weight, and body/organ relative weight; data not shown) or biochemical (blood plasma glucose, urea, and ALT activity) parameters when compared to control group (Table I).

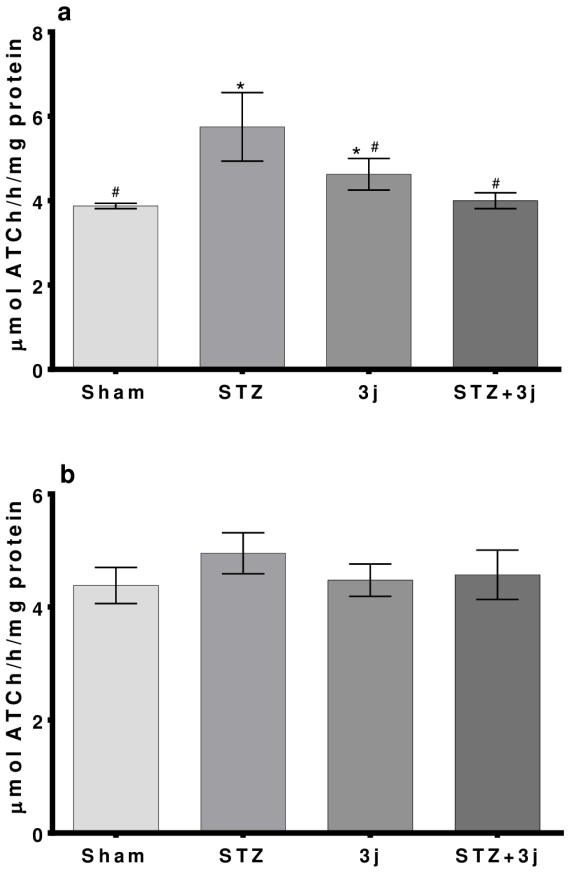


**Figure 5. Hippocampal (a) and cortical (b) AChE specific enzymatic activity of mice treated icv with vehicle or STZ (2.25 mg/kg/day) for 2 days (days 1 and 3) and sc with vehicle or 3j (8.62 mg/kg/day) for 25 days. The results are shown as mean ± standard error (n = 7-8). Duncan's test: different symbols confer significant difference among groups ($p < 0.05$).**

## DISCUSSION

The main objective of this study was to evaluate the possible protective effect of 5'-arylchalcogeno-3-aminothymidine derivative, 3j, against alterations induced by icv-injected STZ. In this context, it is essential to investigate the potentially toxic effects caused by low and high doses of 3j administered for long and short periods in young adult mice.

Mice treated with STZ similarly explored both objects in the NOR behavioral task (known and unknown objects), which indicates that STZ hinders the capacity of recognizing novel and familiar objects. Though 3j did not significantly revert this effect, the STZ-3j group explored the new object similar to the sham group (almost 50% more than the old object). The harmful effect of STZ on cognition is well documented (Shoham et al. 2007, Tota et al. 2009, Javed et al. 2011, Orduña et al. 2011, Biasibetti et al. 2013, Esteves et al. 2017, Wang et al. 2018) and has already been reported by our group (Costa et al. 2016, 2017).

Regarding spatial memory and learning (MWM task), the STZ-3j group presented a higher latency time to reach the refuge site in comparison to the other groups. While the sham animals presented latency on day 3 equivalent to thirty-two percent of the latency presented on day 1, the STZ-animals presented latency equivalent to only fifty-six percent of that demonstrated on the first day (acquisition phase). However, 3j treatment of STZ-mice harmed performance on the test day.

Increased cholinesterase activity leads to cholinergic deficiency, which may have caused memory deficit since the hippocampus plays an essential role in the ability to memorize. Increased AChE by STZ was previously described by our laboratory (Costa et al. 2016, 2017, Thomé et al. 2018) and other authors (Agrawal et al. 2009, Kumar & Singh 2017, Rajasekar et al. 2017, Kumar & Bansal 2018). Our results corroborate this effect of STZ on hippocampal AChE and demonstrate that compound 3j was effective in recovering AChE activity in the hippocampus and improved the cognitive function of STZ-treated animals. On the other hand, enzymatic activity in the cortex remained unchanged in STZ treatment. The compound studied altered the increment in the specific activity of hippocampal AChE. However, the mechanism of this requires additional specific studies, because enzymatic activity was not analyzed immediately after treatment with 3j. The telluric compound may

**Table I. Blood plasma glucose, urea, and ALT activity of mice treated sc with vehicle or 3j during 10 (21.55 or 43.10 mg/kg/day) or 37 days (4.31 or 8.62 mg/kg/day).**

| | Glucose (mg/dL) | Urea (mg/dL) | ALT (U/mL) |
|---|---|---|---|
| **10 DAYS** | | | |
| Sham | 191.8 ± 17.90*# | 61.64 ± 6.585 | 8.460 ± 8.460 |
| 3j 21.55 mg/kg/day | 151.4 ± 3.86* | 65.06 ± 6.316 | 21.924 ± 16.062 |
| 3j 43.10 mg/kg/day | 199.9 ± 11.71# | 64.63 ± 4.067 | 26.250 ± 9.003 |
| **37 DAYS** | | | |
| Sham | 181.9 ± 23.30 | 66.67 ± 4.958 | 26.29 ± 5.205 |
| 3j 4.31 mg/kg/day | 240.2 ± 8.31 | 53.76 ± 4.038 | 22.50 ± 1.360 |
| 3j 8.62 mg/kg/day | 256.3 ± 25.49 | 53.27 ± 4.022 | 23.00 ± 2.964 |

**The results are shown as mean ± standard error (n = 3-5). Different symbols confer significant difference among groups (Duncan's test: $p < 0.05$).**

have prevented or reversed the increase in enzymatic activity. Additionally, STZ-treated mice did not present locomotor impairment in OF, which indicates that this agent does not cause harmful effects in the cerebral region regarding motor function (Ajioka 2016).

Longer treatments may mitigate the harmful effects caused by STZ. In addition to this alternative, preventive treatments with 3j may be tested, as verified by Costa et al. (2017), who reported that N-acetylcysteine mitigated STZ-induced impairments.

The inability of 3j in reversing STZ-induced behavioral impairments cannot be attributed to reduced acetylcholine concentration since the chemical reverted hippocampal AChE activity to normal levels. Farajdokht et al. (2017) and Proença et al. (2021) demonstrated the protective effect of other compounds, troxerutin and purple grape juice, respectively, on hippocampal AChE and oxidative stress. Considering that the telluric organocompound worsened behavioral performance in addition to STZ, this event may affect other neuronal mechanisms and circuits established in the cognitive process and/or memory formation.

Despite 3j not present toxicity in mice, it failed to represent a possible alternative treatment for cognitive deficits such as STZ-induced AD. Compound 3j in the employed sc doses (4.31 or 8.62 mg/kg/day for 37 days and 21.55 or 43.10 mg/kg/day for 10 days) did not cause alterations, such as blood glucose levels, renal and hepatic functions, or apparent alterations, such as in body and organ weight in relation to control group. These results corroborate Rosa et al. (2017), who evaluated 3j toxicity when given as a single 43.10 mg/kg dose to mice.

Thus, our results suggest that this chemical compound does not have pronounced toxicity and may be investigated to pharmacological purposes in the treatment of symptoms and signals of other pathologies where other telluric compounds are effective (Jacob et al. 2000, Zeni et al. 2001a, b, Ávila et al. 2008, 2011, Borges et al. 2008, Okoronkwo et al. 2009). Additionally, 3j could be administered in a daily dose higher than 8.62 mg/kg/day or time interval longer than 25 days in one 8.62 mg/kg/day dose, since, as demonstrated in our results, higher doses and more extended periods are not harmful to young adult mice. Further investigations

with these options are necessary to better understand these possibilities of treatment and exposures to 3j to prevent STZ damages.

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NCP and CSO: performed experimental studies and statistical analysis, and wrote the manuscript; JSB: carried out experimental protocol and statistical analysis; MSCS and SLM: carried out experimental protocol; RSS, RMR and OEDR: synthesized the compound 3j; MEP: supervised the experiments, wrote and revised the final version of the manuscript.



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