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Repeated administration of a selenium-containing indolyl compound attenuates behavioural alterations by streptozotocin through modulation of oxidative stress in mice

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ABSTRACT

Although the pathophysiology of major depression disorder (MDD) is still poorly understood, mounting evidence suggests that the brains of depressed patients are under oxidative stress, leading to depressive symptoms that may include anxiety and cognitive impairment. This study aimed to investigate if the seleno-organic compound 1-methyl-3-(phenylselanyl)-1H-indole (MFSeI) reverses the depression- and anxiogenic-like behaviour, cognitive impairment and oxidative stress induced by the intra-cerebroventricular injection of streptozotocin (STZ; 0.2 mg/4 μl/per mouse) in Swiss male mice. Twenty-four hours after the STZ injection, mice were treated with MFSeI (10 mg/kg, intra-gastrically), or vehicle solution, once daily for seven days. The behavioural tests were performed 30 min after the final MFSeI administration, followed by euthanasia and collection of the cerebral cortex and hippocampus. Administration of MFSeI reversed the depression- and anxiogenic-like behaviour and cognitive impairment induced by STZ, in mice. Neurochemical analyses demonstrated that MFSeI reversed the STZ-increased levels of reactive species, nitrite, lipid peroxidation and acetylcholinesterase activity in the cerebral cortex and hippocampus of mice. Moreover, a single administration of MFSeI (300 mg/kg, intra-gastrically) did not cause acute toxicity in Swiss male mice. Altogether, our data suggest that MFSeI exhibits antidepressant- and anxiolytic-like effects and improves the cognition of STZ-treated mice, without any toxicity.

1. Introduction

Selenium is an essential micronutrient that is most active as a constituent of seleno-proteins that act as oxidoreductases (Brigelius-Flohe and Flohe, 2017). Selenium deficiency has been associated with mood disorders, such as depression and anxiety (Pasco et al., 2012; Wang et al., 2018). Indeed, psychiatric disorders are characterised by increased oxidative stress (Salim, 2017), and, therefore, it is no surprise that several research groups are studying the behavioural and biochemical effects of selenium-containing organic compounds. For example, seleno-organic compounds have been shown to possess antioxidant (Vieira et al., 2017; Vogt et al., 2018), antidepressant-like and anxiolytic-like effects (Domingues et al., 2019), as well as anti-inflammatory and anti-nociceptive actions (Birmann et al., 2018; Rosa et al., 2018), in preclinical studies. Similarly, the indole group has also received extensive attention in preclinical investigations, since it is one of the most widely occurring heterocycles in nature. For example, it is present in the amino acid tryptophan, in the hormone melatonin, and in the neurotransmitter serotonin. In addition, indole derivatives possess antioxidant (Casaril et al., 2017), antidepressant-like (Casaril et al., 2019), antitumour (Rosales et al., 2019) and neuroprotective (Chen et al., 2018) effects.

The intra-cerebroventricular (ICV) administration of streptozotocin (STZ) has been shown to cause neuroinflammation, neuronal loss and oxidative stress in the brain of mouse models, leading to depression- and anxiogenic-like behaviour and symptoms of cognitive impairment (Ishrat et al., 2009; Souza et al., 2013; Zhang et al., 2016). The first line of treatment for depression, *i.e.*, selective serotonin reuptake inhibitors (SSRIs), show considerable adverse effects (such as nausea, insomnia, sexual dysfunction and sleep disturbances) and modest efficacy. It is, therefore, important to find better treatments for depression and to use validated preclinical models to investigate novel antidepressant

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molecules (Lader, 2007; Shelton, 2019).

The involvement of oxidative stress in depression has been investigated for many years; studies have shown that depressed patients have either increased reactive species levels or decreased antioxidant defences (Bajpai et al., 2014; Michel et al., 2012). Of note, it has been shown that 1-methyl-3-(phenylselanyl)-1*H*-indole (MFSeI), an indole- and selenium-containing compound, displays antioxidant activity *in vitro* (Vieira et al., 2017). Considering the promising biological effects of seleno-organic and indole compounds, and the urgent need for better molecules to treat depression, the aim of this study was to investigate if MFSeI can reverse the depression- and anxiogenic-like behaviour, cognitive impairment and oxidative stress induced by STZ, in mice.

2. Materials and methods

2.1. Animals

The behavioural experiments were conducted using adult male *Swiss* mice (25–35 g). All animals were maintained on a 12-h light/12-h dark cycle (lights turned on at 7:00 a.m.). The environment temperature (22–25 °C) was kept constant. Mice were housed in cages with free access to food and water. The animals were maintained in regular cages, with 5 animals per cage. The experiments were performed according to a randomized schedule and each group of animals was used in determined tests and a separate group was used to euthanasia. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. The present experimental study was approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Pelotas, Brazil (6305-2017). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

The MFSeI (Fig. 1A) was prepared and characterised in the Laboratory of Clean Organic Synthesis at the Federal University of Pelotas, Brazil. The MFSeI was dissolved in canola oil and administered intragastrically (i.g.) at a dose of 10 mg/kg. The dose of based in the previous studies not published yet. Fluoxetine hydrochloride (Pfizer, Brazil) was diluted in saline and administered at dose 10 mg/kg (i.g.) (Shafia et al., 2017). All drugs were administered at a constant volume of 10 ml/kg.

I.g. procedure is commonly used by our research group for administration of organic compounds and oil-soluble drugs, in this method compounds are administered by using a gastroesophageal probe that releases them directly into the stomach. The i.g. is route was chosen because is more common used in the laboratories (Casaril et al., 2019; Domingues et al., 2018; Fronza et al., 2017) to administration of compounds and this route mimes the oral route used by human and it is more close to use in the clinical study futures.

STZ was purchased from Sigma-Aldrich. (St Louis USA), dissolved in saline 0.9% and administered by ICV injection (0.2 mg/4 µl/per mouse). All other chemicals were of analytical grade and obtained from standard commercial suppliers. STZ could be easily disassembled, but a STZ solution pH 3 is impossible administer ICV in mice; it could cause a severe toxicity. Furthermore, with purpose to reduce disassemble we take care about to maintain in low temperatures and in the dark during the experiment. Dose and the administration time of STZ were selected based on our pilot studies and previously published data (Amiri et al., 2017; Pinton et al., 2011; Souza et al., 2017a).

2.3. Experimental protocol

The experimental design is illustrated in Fig. 1B. Three different sets of mice underwent the same treatment regimen (n = 6 mice/group): First, mice received STZ or saline (vehicle) through ICV injection. After 24 h, the animals received MFSeI (10 mg/kg), fluoxetine (10 mg/kg) or vehicle (canola oil) once daily for seven consecutive days. Thirty minutes after the last treatment, the behavioural tests were performed. The first set of mice was tested in the open-field test, elevated plus maze and forced swimming test, and the second set of mice was tested in the social interaction test, Y-maze and splash test. The third set of mice was

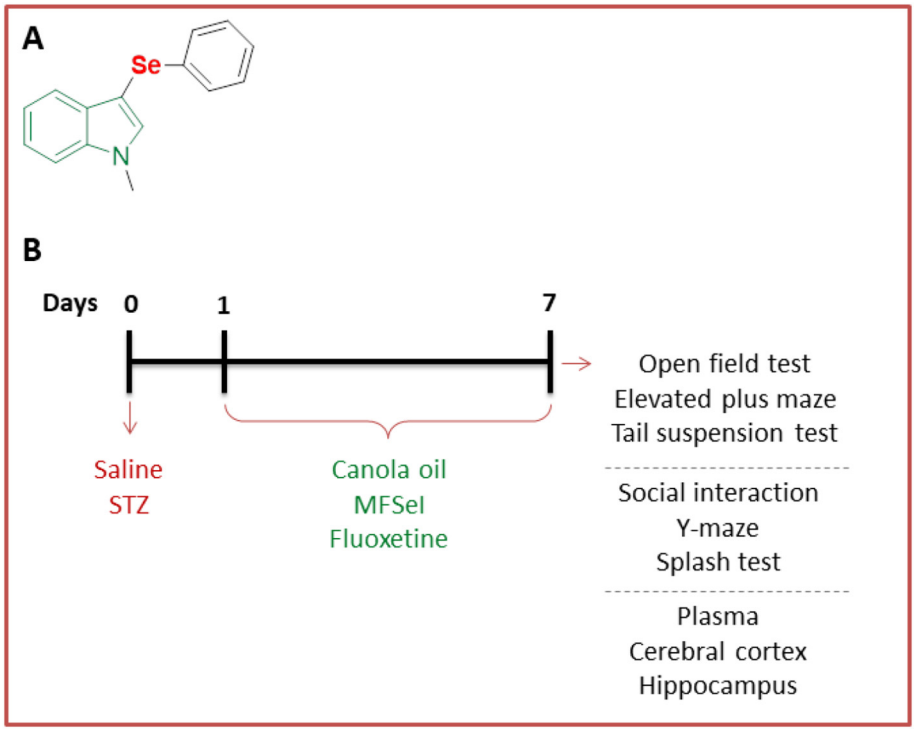

Fig. 1. Overview of the present study. (A) Chemical structure of 1-methyl-3-(phenylselanyl)-1H-indole (MFSeI). (B) Schematic representation of the experimental employed. STZ: streptozotocin.

euthanized for collection of plasma and removal of cerebral cortex and hippocampus.

In this current study we choose use separate groups of animals for different behavioural tests and for euthanasia. We have some explanation for this choice. First, we used six behavioural tests, and to reduce the stress of animals and the to remove the possible interaction the animals between tests were separate in two sets, one set to open fiend test, elevated plus maze and tail suspension test and the another set to social interaction, Y-maze and splash test. The third set of animals was used to *ex vivo* analyses.

It is important highlight that the first and second sets of animals were euthanized after the end of behavioural tests that occurs exactly 30 min after the last treatment with MFSeI and fluoxetine. The third set used to *ex vivo* analysis was euthanized 30 min after the last treatment too. Immediately after euthanasia the samples of prefrontal cortex, hippocampus and plasma were excised and stored at −80 °C for *ex vivo* analyses.

2.4. ICV injection

For the ICV injection, mice were anaesthetized by isoflurane (Iso Fo Abbott Laboratories) inhalation. The ICV injection was performed through "free-hand" method, as previously described by Haley and McCormick (1957) and modified by Laursen and Belknap (1986), using the bregma fissure as a reference point. For this method, a microsyringe (25 µl, Hamilton) was inserted perpendicularly at 0.8 mm posterior to bregma, 1.0 mm lateral to sagittal suture, and 3.0 mm beneath the surface of the brain. The needle was inserted unilaterally 1 mm into the midline point equidistant from each eye at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. The animals were immobilized and a gauze soaked in 70% ethanol was utilized for the asepsis of the injection site. The injection was given over 30 s, and the needle was kept in place for a further 30 s to avoid reflux of the injected solution.

2.5. Behavioural tests

2.5.1. Open field test (OFT)

With the purpose of excluding sedative or motor abnormality, the mice were tested in the open field test. The mice were placed in the center of a wooden box (30 × 30 × 15 cm) divided into nine squares of equal areas. During a 5 min session, the number of groomings (as an indication of anxiety) and the locomotor (through the number of crossed squares) and exploratory (number of elevations) activity were evaluated (Walsh and Cummins, 1976). The apparatus was cleaned with a solution of 70% ethanol between tests in order to hide animal clues.

2.5.2. Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice (acoustically and visually isolated) were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail during 6 min. Mice were considered immobile only when they hung passively and completely motionless. Immobility time was manually recorded during the last 4-min period.

2.5.3. Splash test

The splash test was carried out as described by Detanico et al. (2009) and Pesarico et al. (2016). The grooming behaviour was observed as a measurement of motivational and self-care difficulties. The test consisted of squirting a 10% sucrose solution on the dorsal coat of a mouse placed individually in a cage. The total grooming activity (including nose/face grooming, head washing and body grooming) was recorder for 5 min.

2.5.4. Social interaction test

The social interaction test was realized in the same apparatus that was used for the OFT and it was performed based on previous data showing that mice with depressive-like behaviour spend less time interacting with an unknown mouse (Gupta et al., 2016). The intruder mice were the same age, sex, weight and unknown to the test mouse. For the test, the test mouse and the intruder mouse were placed on different sides of the open field. The social interaction behaviour was recorded during a 5 min session and consisted of grooming, mounting and crawling under the intruder mouse, including the passive interaction (number of crossing to the stimulus mouse). Manifestation of an aggressive behaviour from either mice resulted in interruption of the test.

2.5.5. Y-maze test

Y-*maze* is used to assess memory function as spatial short-term memory based on the willingness of mice in exploring new environments (Sarter et al., 1988). The Y-maze is a three-arm horizontal maze (40 cm long and 3 cm wide with walls 12 cm high) in which the three arms are symmetrically separated at 120°. Mice were initially placed in one arm (A), and the arm entry sequence (*e.g.* ABCACB, where letters indicate arm codes) and the number of arm entries was recorded manually for each mouse over a 5 min period. The entry was defined as placing all four paws within the boundaries of arm. The alternation was defined as entries into all three arms consecutively (*i.e.* ABC, BAC or CBA but not BAB) without repetition. The following formula determined percentage alternation: % Alternation = [(Number of alternations × 3)/(Total arm entries - 2)] × 100.

2.5.6. Elevated plus maze test

Anxiolytic-like behaviour was accomplished using the elevated plus maze task as previously described (Frussa-Filho et al., 1999; Rubin et al., 2000). The apparatus consists of a wooden structure raised to 50 cm from the floor. This apparatus is composed of four arms of the same size, with two closed-arms (walls 40 cm) and two open-arms connected by a central platform. Initially, the animals were placed on the central platform of the maze in front an open arm. An entry was defined as placing all four paws within the boundaries of the arm. The mice had 5 min to explore the apparatus, and the time spent and the number of entries in open and closed-arms were recorded.

2.6. Tissue preparation

The separate group of animals was euthanized and immediately the samples of prefrontal cortex, hippocampus and plasma were excised and stored at −80 °C for *ex vivo* analyses. The cerebral cortex and hippocampus were separated in two hemispheres in order to submit each sample to all analyses. The brain hemispheres were homogenized in 50 mM Tris-HCl, pH 7.4 (1:4, w/v) for the determination of the levels of reactive species (RS), nitrite metabolites and thiobarbituric acid reactive species (TBARS). To evaluate the acetylcholinesterase (AChE) activity prefrontal cortex and hippocampus were homogenized in 25 mM sucrose buffer (1:10, w/v).

2.6.1. Reactive species quantification

The quantification of RS was performed according Loetchutinat et al. (2005). In this assay, dichlorodihydro-fluorescein diacetate (DCHF-DA) oxidation in to fluorescent dichlorofluorescein (DCF) is evaluated for intracellular RS detection. The fluorescence intensity was recorded in spectrofluorophotometer in 520 nm emission and 480 nm excitation and results were expressed as arbitrary units of fluorescence.

2.6.2. Nitrite production

The accumulation of nitrite, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Griess reagent (0.1% N-[1-naphthyl] ethylene diamine dihydro chloride, 1%

sulfanilamide and 2.5% phosphoric acid) (Beda and Nedospasov, 2005). Absorbance was determined at 550 nm and the concentration of nitrite was expressed as μmol/mg protein.

2.6.3. Thiobarbituric acid reactive species assay

TBARS levels were estimated by the amounts of malondialdehyde (MDA) formed using the thiobarbituric acid (TBA) reagent (Ohkawa et al., 1979). Submission of samples to high temperatures favours the binding of TBA to MDA, forming a pink chromophore that was measured spectrophotometrically at 532 nm. The results were expressed as nmol MDA/g tissue.

2.6.4. Acetylcholinesterase activity

Assessment of AChE activity was carried out using dithiobis-2-nitrobenzoate (DTNB) (Ellman reagent) as described by Ellman et al. (1961) using acetylthiocholine as substrate. In this assay, thiocholine (8 mM, acetyl choline analog) complexed to DTNB 10 mM reduced DTNB to 5-thio-2-nitro-benzoic acid (TNB). TNB has a yellowish color proportional to AChE activity. The change in absorbance was measured spectrophotometrically immediately at zero and 1 min at 412 nm. The AChE activity in the supernatant was expressed as nmol/min/mg protein.

2.7. Plasma glucose levels

Glucose levels were measured in the plasma of mice by an enzymatic colorimetric method using a commercial kit (Labtest, Diagnostica S.A., Minas Gerais, Brazil). Glucose levels were expressed as mg/dl.

2.8. Acute toxicity and biochemical parameters

The determination of acute toxicity biomarkers were performed using two groups of adult male *Swiss* mice (n = 6 mice/group): canola oil (vehicle, i.g.) and MFSeI (300 mg/kg, i.g.).

Observations were carried out daily during the first 72 h after the treatment to look for any clinical signs of toxicity, like changes in fur, skin, eyes and nasal secretions (mucus and bleeding), incidence of secretions and excretions, lacrimation, piloerection and unusual respiratory pattern. After 72 h, mice were anaesthetized (inhalation of isoflurane) for blood collection by cardiac puncture followed by euthanasia by cervical dislocation for the removal of brain, liver and kidney.

Blood was collected and centrifuged at 2000 ×*g* for 10 min to obtain the plasma, which was used for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT) activity, glucose levels, lactate dehydrogenase (LDH), urea and creatinine. The total brain, liver and kidney were homogenized in 50 mM Tris-HCl, pH 7.4 (1:10, w/v), and the homogenate was centrifuged at 2500 ×*g* for 10 min at 4 °C and was used for the measurement of RS, TBARS, δ-aminolevulinate dehydratase (δ-ALA-D) activity. RS and TBARS levels were assessed as previously described.

2.8.1. δ-aminolevulinic dehydratase activity

δ-ALA-D is a sulfhydryl-containing and Zn^{2+}-containing enzyme and highly sensitive to prooxidants and heavy metals. The δ-ALA-D activity was assayed according to the method of Sassa (1982) by measuring the rate of porphobilinogen (PBG) formation. An aliquot of S1 was incubated for 0.5 h (liver), 1 h (kidney) and 3 h (brain) at 37 °C. The reaction was stopped by addition of trichloroacetic acid (TCA). The reaction product was determined using modified Ehrlich's reagent at 555 nm. δ-ALA-D activity was expressed as nmol PBG/mg protein/h.

2.8.2. Biochemical parameters

Plasmatic AST, ALT, glycemia, LDH, urea and creatinine were quantified using commercial kits (LABTEST, Diagnostica SA, Brazil), the results were expressed as conventional units (mg/dL).

2.9. Protein quantification

Protein quantification was performed using assay of Bradford (1976) measuring the formation of a blue color at a wavelength of 595 nm. Bovine serum albumin (BSA) was used as the control.

2.10. Statistical analysis

Data from behavioural and neurochemical experiments of mice challenged with STZ and treated with MFSeI and fluoxetine were analyzed by one-way analysis of variance (ANOVA), followed by the Newman-Keuls *post hoc* test. Data from toxicological evaluation were analyzed by Student *t*-test. All data are represented as mean ± standard error of the mean (S.E.M.) and were analyzed by GraphPad Prism 7.0. Values < 0.05 ($p \leq 0.05$) were considered statistically significant.

3. Results

3.1. Plasma glucose levels

The blood glucose levels were the same in all experimental groups ($F_{(5,30)} = 0.569$; $p = 0.72$) (data not shown).

3.2. MFSeI treatment abolishes the depressive-like behaviour induced by STZ

Fig. 2A–D shows the immobility time of mice in the TST, grooming time in the splash test, duration of social interaction and number of social interactions in the social interaction test, respectively.

STZ increased the immobility time in TST (Fig. 2A), compared to the control group; MFSeI and fluoxetine were effective against this increase ($F_{(5,30)} = 12.4$; $p < 0.001$). In the splash test, STZ decreased the grooming time (Fig. 2B), compared to the control group; treatment with MFSeI and fluoxetine increased the grooming time induced by STZ ($F_{(5,30)} = 13.7$; $p < 0.001$).

Both treatments, MFSeI and fluoxetine, reversed the depressive-like behaviour induced by STZ, for duration of social interaction ($F_{(5,30)} = 6.7$; $p < 0.001$) (Fig. 2C) and number of social interactions ($F_{(5,30)} = 3.81$; $p = 0.009$) (Fig. 2D).

3.3. MFSeI treatment improves the anxiogenic-like behaviour and deficit cognition induced by STZ

Mice subjected to STZ spent more time at grooming behaviour (Fig. 3A), compared to the control group. The time spent grooming decreased in mice treated with MFSeI, but not fluoxetine ($F_{(5,29)} = 5.14$; $p = 0.002$). The grooming behaviour observed in the open-field box represented anxious behaviour.

STZ decreased the time spent on open arms (Fig. 3B) and reduced the number of open arm entries (Fig. 3C), compared to the control group; both MFSeI and fluoxetine treatment reversed this alteration in the EPM test ($F_{(5,30)} = 12.4$; $p < 0.001$).

Treatment by MFSeI and fluoxetine reversed the decrease in the number of alternations in the Y-maze test, induced by STZ administration ($F_{(5,29)} = 7.24$; $p < 0.001$) (Fig. 3D).

3.4. MFSeI effects on locomotor activity of mice exposed to STZ

Fig. 4 shows the effects of treatment with MFSeI and fluoxetine on locomotor activity of mice exposed to the STZ. Neither STZ nor treatment by MFSeI or fluoxetine changed the numbers of crossings (Fig. 4A) ($F_{(5,30)} = 0.737$; $p = 0.60$) and rearings (Fig. 4B) ($F_{(5,30)} = 0.396$; $p = 0.85$) by mice.

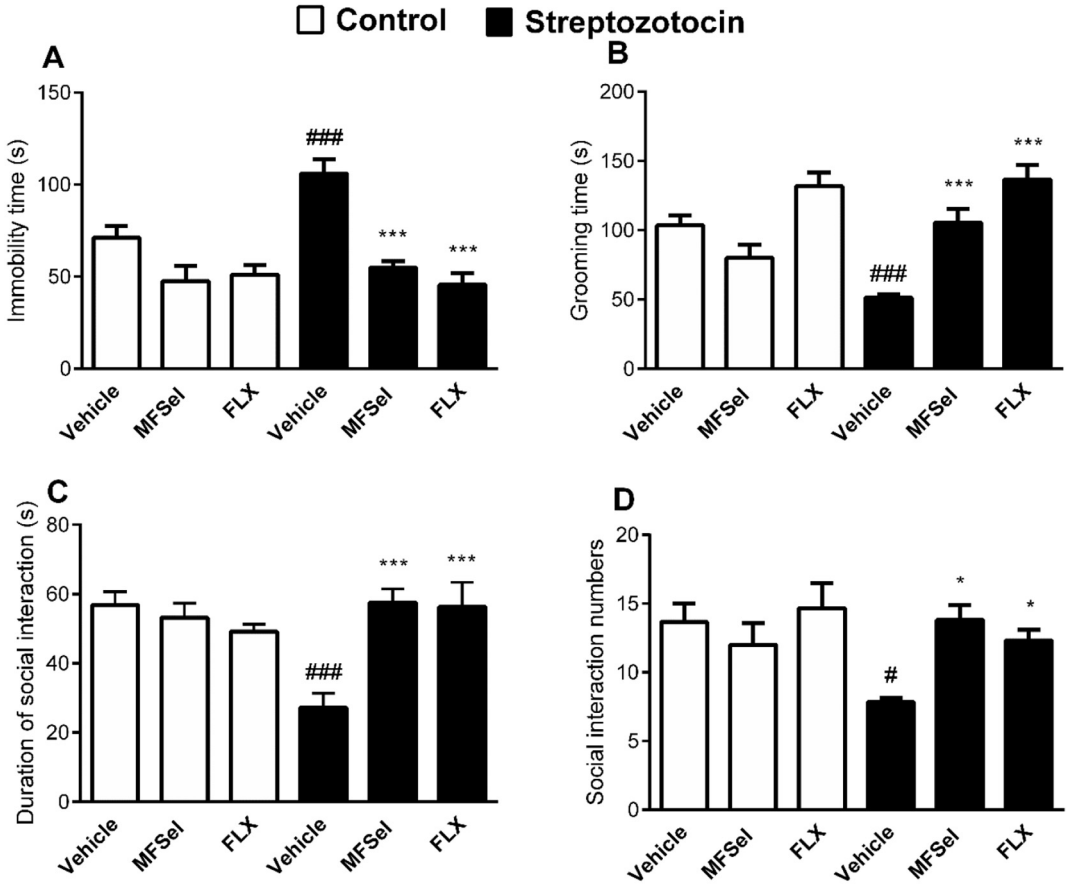

Fig. 2. The MFSeI and fluoxetine effect on immobility time in the tail suspension test (A), grooming time in the splash test (B), duration of interaction social (C) and number of interaction social (D) in the interaction social test in a mouse model induced by STZ. Data are shown as means ± S.E.M. of 6 animals per group. $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ compared to the control group; $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to the STZ group (one-way ANOVA followed by the Newman-Keuls). MFSeI: 1-methyl-3-(phenylselanyl)-1*H*-indole; FLX: fluoxetine;

3.5. Involvement of oxidative stress

Fig. 5A and B show that administration of STZ increased lipid peroxidation levels in the prefrontal cortex ($F_{(5,30)} = 4.78$; $p = 0.002$) and hippocampus ($F_{(5,30)} = 5.66$; $p < 0.001$) of mice, compared to the control group. MFSeI and fluoxetine reversed the lipid peroxidation levels induced by STZ, in both structures.

Fig. 5C demonstrates that STZ increased RS levels in the prefrontal cortices of mice; both MFSeI and fluoxetine treatment abolished this increase ($F_{(5,30)} = 6.35$; $p < 0.001$). The same results occurred in the hippocampus (Fig. 5D), and both treatments were effective in reversing the increase induced by STZ ($F_{(5,30)} = 5.04$; $p = 0.002$).

Nitrite production is an indirect measurement of nitric oxide. The results show an increase in the production of nitrite induced by STZ, compared to the control group, and this effect was reversed by MFSeI and fluoxetine treatment in the prefrontal cortex (Fig. 5E) ($F_{(5,30)} = 8.74$; $p < 0.001$) and hippocampus (Fig. 5F) ($F_{(5,30)} = 7.05$; $p < 0.001$).

3.6. Involvement of the cholinergic system

As shown in Fig. 6A and B, STZ increased AChE activity, compared to the control group, in the prefrontal cortex ($F_{(5,20)} = 4.85$; $p = 0.005$) and in the hippocampus ($F_{(5,22)} = 4.48$; $p = 0.006$), respectively. Furthermore, MFSeI and fluoxetine attenuated STZ-induced AChE activity.

3.7. MFSeI has no toxic effect on blood and biochemical parameters

After administration of MFSeI (300 mg/kg) no clinical signs of toxicity and no deaths were observed. Tables 1 and 2 show that there was no alteration of toxicity biomarkers in the brain, kidney, liver and heart of mice.

4. Discussion

The results of the present study clearly show that MFSeI treatment exhibits a significant antidepressant-like and anxiolytic-like effect and reverses cognitive impairment induced by STZ. In terms of a theoretical mechanism of action for MFSeI, this study shows that this seleno-organic compound reverses the STZ-induced increase in RS, nitrite, and TBARS levels, and AChE activity, in the cerebral cortex and hippocampus of mice. Importantly, MFSeI treatment did not affect the locomotor and exploratory behaviours and did not produce any signs of acute toxicity, in the mice, suggesting the treatment is safe to administrate.

Previous studies have demonstrated that ICV injection of STZ causes depression- and anxiogenic-like behaviours, and memory impairment, in rodents (Amiri et al., 2017; Grieb, 2016; Souza et al., 2013), making it useful for research into novel antidepressant molecules. Accordingly, we replicated these findings, as we found that a single injection of STZ induced behavioural and biochemical alterations in mice, which allowed us to investigate the potential pharmacological effects of a novel synthetic organo-selenium compound. The antidepressant-like effect of MFSeI was observed in its ability to decrease immobility time in the

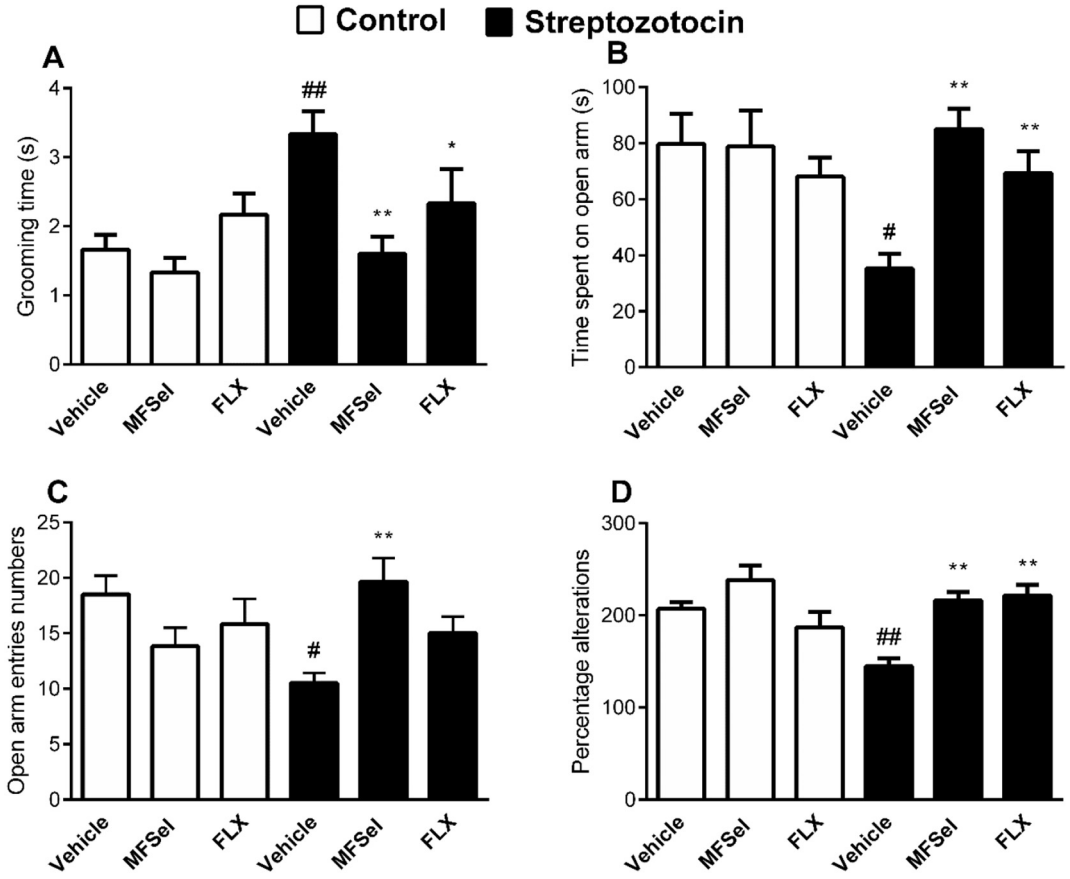

Fig. 3. The MFSeI and fluoxetine effect on grooming time in the open-field box (A), time spent on open arm (B) and number of open arm entries (C) in the EPM test and percentage alternations in Y-maze test (D) in a mouse model induced by STZ. Data are shown as means ± S.E.M. of 6 animals per group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the STZ group (one-way ANOVA followed by the Newman-Keuls). MFSeI: 1-methyl-3-(phenylselanyl)-1*H*-indole; FLX: fluoxetine;

TST, increase grooming time in the splash test and increase interaction time in the social interaction test, in STZ-treated mice. Furthermore, the ability of MFSeI to reverse the decreased number of entries and the decreased time spent in the open arms in the EPM, induced by STZ, suggests that MFSeI possesses anxiolytic-like effects. MFSeI administration reversed the decrease in the number of spontaneous alterations induced by STZ in the Y-maze, indicating that this seleno-organic compound can also improve cognition.

In addition to the behavioural effects, MFSeI treatment also restored neurochemical alterations induced by STZ. Previously, studies have suggested that an increase in oxidative stress in the brain may play a role in the pathogenesis of depression and that antidepressant drugs modulate this imbalance (Eren et al., 2007; Moylan et al., 2014). In agreement with this, ICV injection of STZ alters the proinflammatory cytokine pathways and brain-derived neurotrophic factor (BDNF) kynurenine and tryptophan levels (Souza et al., 2017a; Souza et al.,

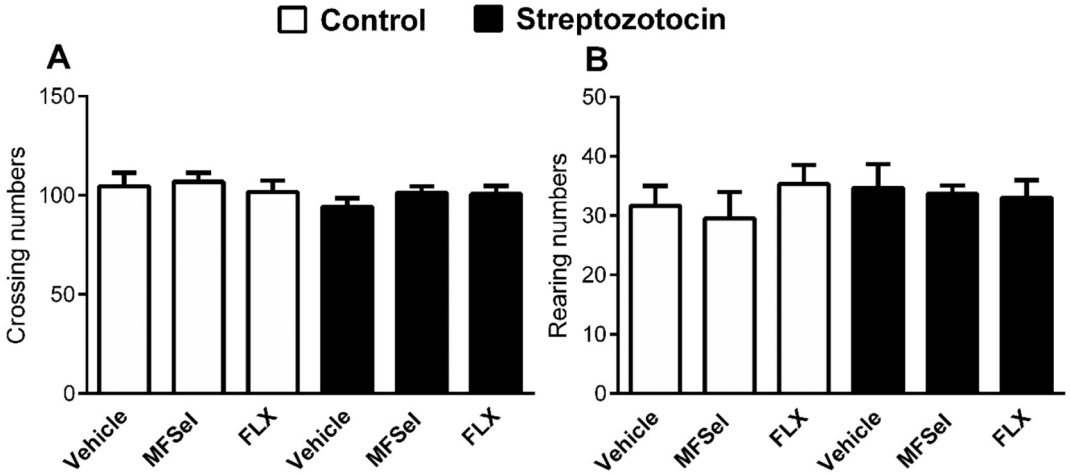

Fig. 4. The MFSeI and fluoxetine effect on crossing numbers (A) and rearing mumbers (B) in the open-field test in a mouse model induced by STZ. Data are shown as means ± S.E.M. of 6 animals per group. (one-way ANOVA followed by the Newman-Keuls). MFSeI: 1-methyl-3-(phenylselanyl)-1*H*-indole; FLX: fluoxetine;

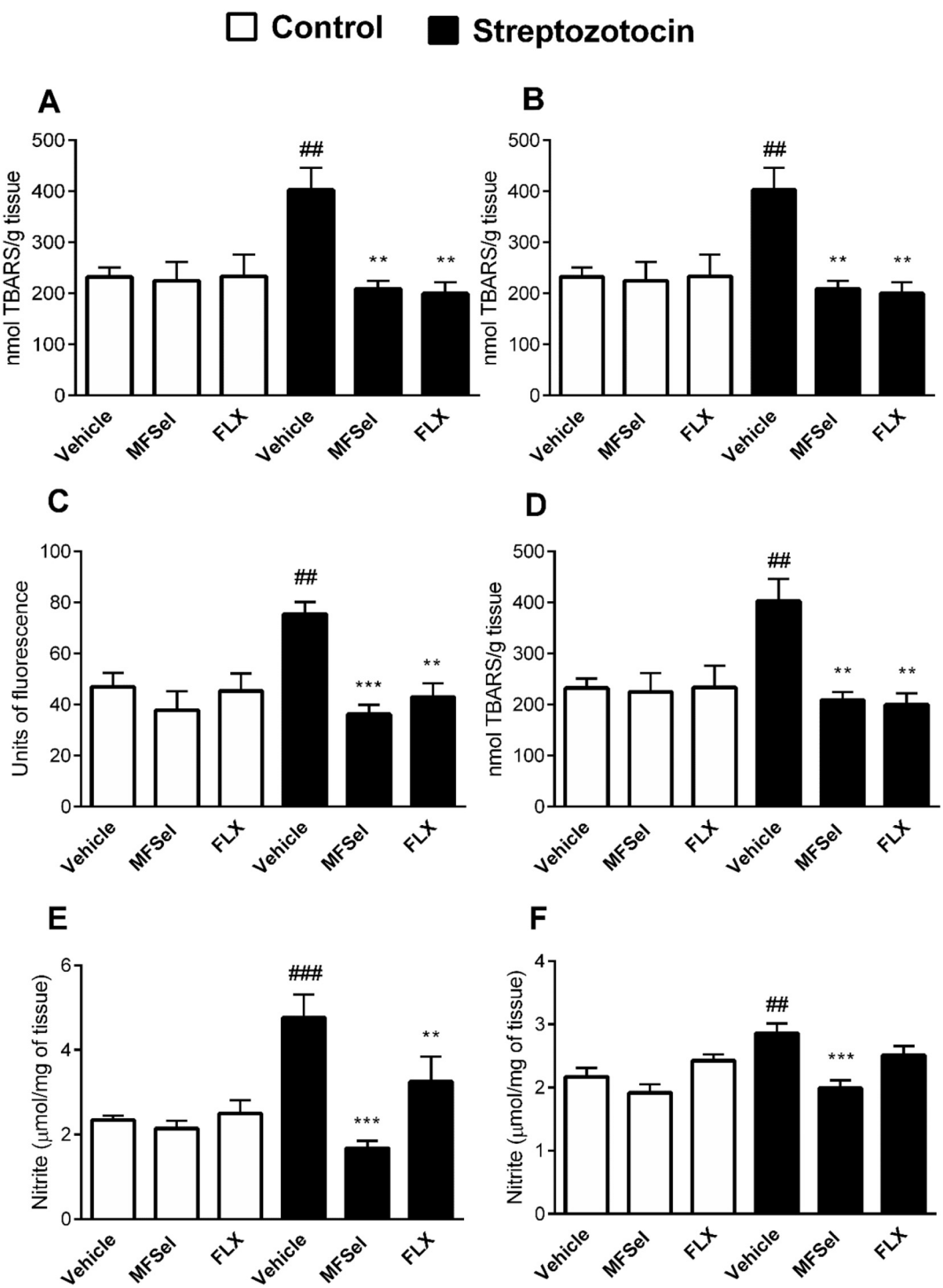

Fig. 5. The MFSeI and fluoxetine effect on the TBARS levels of prefrontal cortex (A) and hippocampus (B), on the RS levels of prefrontal cortex (C) and hippocampus (D) and on the nitrite levels of prefrontal cortex (E) and hippocampus (F) in a mouse model induced by STZ. Data are shown as means ± S.E.M. of 6 animals per group. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.01$ compared to the control group; $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the STZ group (one-way ANOVA followed by the Newman-Keuls). MFSeI: 1-methyl-3-(phenylselanyl)-1*H*-indole; FLX: fluoxetine;

2017b), leading to oxidative damage (Thome et al., 2018). Analyses of the oxidative stress parameters demonstrated that MFSeI reversed the increased levels of RS, nitrite and TBARS in the cerebral cortex and hippocampus of mice treated with STZ. In this way, STZ administration caused lipid peroxidation that was abolished by treatment with MFSeI, indicating the involvement of antioxidant effects in the decrease of oxidative stress caused by the STZ protocol. Accordingly, MFSeI has been reported to have antioxidant properties *in vitro* (Vieira et al., 2017). According to our results, the MFSeI antidepressant- and anxiolytic-like action and cognition improvements are related to its effectiveness in maintaining anti-oxidative homeostasis in the prefrontal cortices and hippocampus of mice.

In addition, some studies have demonstrated that RS might be generated during nitrite metabolism, as a breakdown product of nitric oxide (Abdel-Wahab and Moussa, 2019; May et al., 2004). In this study, we found that nitrite levels were increased by STZ administration, and

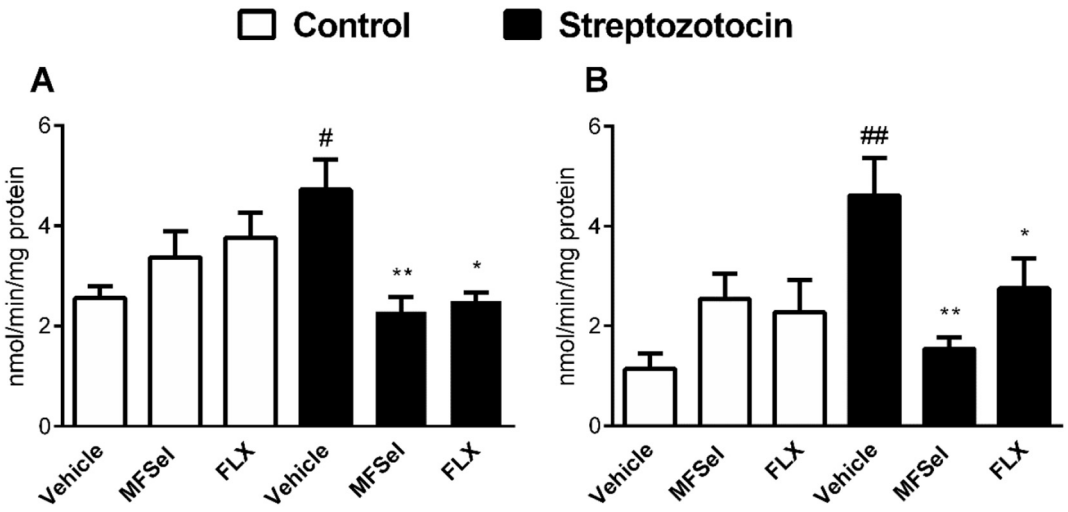

Fig. 6. The MFSeI and fluoxetine effect on the AChE activity of prefrontal cortex (A) and hippocampus (B) in a mouse model induced by STZ. Data are shown as means ± S.E.M. of 6 animals per group. $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.01$ compared to the control group; $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to the STZ group (one-way ANOVA followed by the Newman-Keuls). MFSeI: 1-methyl-3-(phenylselanyl)-1*H*-indole; FLX: fluoxetine;

Table 1
Effect of acute administration of MFSeI (300 mg/kg; i.g.) in toxicology parameters in the plasma of mice.

Analysis	Control	MFSeI	*p*	t
AST	80.20 ± 2.97	69.10 ± 9.75	0.29	1.10
ALT	133.00 ± 4.31	146.00 ± 19.00	0.51	0.67
Glycemia	136.00 ± 8.87	141.00 ± 12.10	0.75	0.32
LDH	14.80 ± 3.41	14.20 ± 2.51	0.87	0.15
Urea	51.60 ± 1.51	45.30 ± 3.14	0.10	1.80
Creatinine	0.10 ± 0.07	0.09 ± 0.06	0.74	0.32

AST and ALT activities were expressed in U/L. Glycemia levels was expressed in mg/dL. LDH was expressed in U/L. Urea and creatinine levels were expressed in mg/dL. Results are expressed as mean ± SEM. ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: Lactic dehydrogenase; MFSeI: 1-methyl-3-(phenylselanyl)-1*H*-indole.

Table 2
Effect of acute administration of MFSeI (300 mg/kg; i.g.) in toxicological parameters in brain, liver and kidney of mice.

Analysis	Control	MFSeI	*p*	t
RS				
Brain	72.80 ± 3.42	75.20 ± 7.60	0.77	0.29
Liver	304.00 ± 11.90	295.00 ± 6.46	0.98	0.01
Kidney	245.00 ± 12.00	245.00 ± 12.00	0.53	0.64
TBARS				
Brain	34.10 ± 2.61	36.40 ± 4.01	0.64	0.46
Liver	37.80 ± 5.08	30.40 ± 6.72	0.39	0.88
Kidney	42.00 ± 9.03	43.30 ± 4.80	0.90	0.12
δ-ALA-D				
Brain	3.20 ± 0.52	2.82 ± 0.28	0.57	0.88
Liver	10.10 ± 0.92	7.68 ± 1.85	0.24	1.23
Kidney	3.80 ± 0.75	4.72 ± 0.45	0.31	1.05

RS quantification was expressed in units of fluorescence. TBARS levels were expressed in nmol MDA/g tissue. δ-ALA-D activity was expressed in nmol PBG/mg/protein/h. Results are expressed as mean ± S.E.M. RS: reactive species; TBARS: thiobarbituric acid reactive species; MDA: malondialdehyde; δ-ALA-D: δ-aminolevulinate dehydratase.

MFSeI and fluoxetine treatment decreased these levels, corroborating the results of oxidative stress; this suggests that the increase in RS levels is due to an increase in nitrite levels.

In the present study, the repeated administration of MFSeI and fluoxetine reversed the STZ- increased activity of AChE in the cerebral cortex and hippocampus of mice. AChE inhibitors (such as donepezil, galanthamine and rivastigmine), which prevent the hydrolysis of acetylcholine, reduced the cognitive decline in neurodegenerative conditions. Deficit in the cholinergic system is one of the major neuropathological characteristics associated with memory loss (Grossberg, 2003; Hasselmo, 2006). Therefore, the reduced cognitive impairment associated with MFSeI administration, observed in the Y-maze test, may be a consequence of its ability to modulate the cholinergic system.

Because of the promising effects of MFSeI as a therapeutic agent, we investigated the possibility of toxicological effects following administration. A dose 10 times higher than the effective dose on behavioural and neurochemical parameters (Casaril et al., 2017) was chosen to rule out the possibility of toxicity for MFSeI. The absence of increased circulating biomarkers of renal, hepatic and cardiac toxicity, together with the absence of altered RS and TBARS levels and δ-ALA-D activity in the brain, liver and kidney of mice, suggests that MFSeI does not induce toxicity in mice, following administration. These results have encouraged us to continue investigating the pharmacological effects of this organo-selenium compound.

It is possible that the activity of MFSeI is closely related to its chemical structure. The indole nucleus is one of the most commonly occurring heterocycles, and is present in the structures of tryptophan, melatonin and serotonin. Additionally, the redox potential of selenoproteins in the brain highlight the importance of controlling RS generation and neutralization. In this sense, molecular hybridisation involving the indole nucleus and the selenium atom is a potential approach in the development of synthetic molecules with promising biological effects under various conditions.

The current study provides preliminary results on the effectiveness of repeated MFSeI treatment on depression, anxiety and cognitive deficit, but further investigations are needed into the applicability of MFSeI, compared to SSRIs. Presently, we are not able to recommend MFSel over SSRI, but the fact that low doses of MFSel were effective could reduce the risk of any undesirable side-effects.

We acknowledge some limitations of this study: (1) the lack of further investigations into the involvement of the cholinergic nervous system, *e.g.*, antagonists of acetylcholine receptors; (2) the fact that only oxidative stress was evaluated, and there were no experiments investigating the neuroprotective effect involved in depressive-like behaviour. To address these limitations, we will conduct further studies to show the involvement of other pathways in the mechanism of MFSeI.

5. Conclusion

The present study revealed that MFSeI abolishes the depressive-like, anxiogenic-like behaviour and cognitive impairment induced by STZ injection in mice. The behavioural effects of this seleno-organic compound appear to involve the reduction of oxidative stress (evidenced by decreased ROS, NO_x and TBARS levels) and AChE activity in the PFC and HC of mice treated with STZ. Importantly, toxicity biomarkers were not altered after MFSeI treatment, demonstrating the safety of administrating this compound. Overall, the results highlight the potential biological effects of a novel selenium-containing compound endowed with antioxidant activity, which could be useful in the treatment of disorders characterised by oxidative stress, such as depression and anxiety. Further studies are being carried out to investigate the effects of MFSeI in different animal models.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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