Journal of Trace Elements in Medicine and Biology 53 (2019) 34–40

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Toxicology

Organoselenotriazoles attenuate oxidative damage induced by mitochondrial dysfunction in *mev-1 Caenorhabditis elegans* mutants

Ana Thalita Gonçalves Soares[a], Luiz Brasil Lopes Rodrigues Junior[a], Willian Goulart Salgueiro[a], Ana Helena de Castro Dal Forno[a], Cristiane Freitas Rodrigues[a], Manoela Sacramento[c,d], Jeferson Franco[b], Diego Alves[c,d], Riva de Paula Oliveira[e], Simone Pinton[f], Daiana S. Ávila[a,*]

[a] *Programa de Pós-Graduação em Bioquímica, Laboratório de Bioquímica e Toxicologia em Caenorhabditis elegans (GBTOXce), Universidade Federal do Pampa, UNIPAMPA, Uruguaiana, RS 97500-970, Brazil*
[b] *Interdisciplinary Center for Biotechnology Research, CIPBIOTEC, Universidade Federal do Pampa, Campus São Gabriel, 97.300-000, São Gabriel, RS, Brazil*
[c] *Programa de Pós-Graduação em Química (PPGQ), Laboratório de Síntese Orgânica Limpa-LASOL, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, UFPel, Pelotas, RS, Brazil*
[d] *Programa de Pós-Graduação em Biotecnologia (PPGB), Grupo de Pesquisa em Neurobiotecnologia-GPN, Biotecnologia/Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas, Pelotas, RS, Brazil*
[e] *Departamento de Biologia Celular e Genética, Universidade Federal do Rio Grande do Norte, Natal, Brazil*
[f] *Universidade Federal do Pampa - Campus Uruguaiana, Uruguaiana, RS, Brazil*

ARTICLE INFO

Keywords:
Selenium
Oxidative stress
Selenotriazoles
Mitochondria
Caenorhabditis elegans

ABSTRACT

Organic selenium compounds have several pharmacological activities already described, as anti-inflammatory and antitumor activities, which have been attributed to their antioxidant effects. Because they are promising in pharmacology, the synthesis of these compounds has increased significantly. As many new molecules are synthesized the use of a simple model like *Caenorhabditis elegans* is highly advantageous for initial evaluation of the toxicity and therapeutic potential of these molecules. The objective of this study was to evaluate the toxicity and antioxidant capacity of a series of selenotriazoles compounds in *C. elegans*. The animals were exposed to the compounds in liquid medium for only 30 min at the first larval stage (L1). The compounds had no toxic effects at the concentrations tested. Treatment with selenotriazoles (10 μM) partially reversed the stress induced by the pesticide paraquat (1 mM). Se-Tz Ia compound partially increased the survival of worms treated with H_2O_2 (0.5 mM). The compounds also increased the longevity of *mev-1* mutants, which have a reduced life span by the production of excessive reactive oxygen species (ROS) in the mitochondria caused by a mutation in complex II of the electron transport chain. In addition, the compounds reduced the levels of ROS determined by the fluorescent probe DCF-DA as well as also reduced catalase enzyme activity in these animals. Based on the results found, it is possible to conclude that the compounds have antioxidant activity mainly in oxidative stress condition generated by a mitochondrial dysfunction in *C. elegans*.

1. Introduction

Oxidative stress is induced by an imbalance of the redox state through the dysfunction in the antioxidant system or by an excessive generation of free radicals. It is present in several pathologies such as neurodegenerative, metabolic and cardiovascular diseases [1,2]. Hence, antioxidant therapy is described as a strategy to treat or delay the development of these diseases.

Mitochondrion is the cellular source for free radical generation due to electron transporter chain, wherein complexes I and III are considered the prime locations for superoxide production. Agents that impair the electron transport chain and/or ATP synthesis have been implicated with the onset of diseases as Parkinson's disease. For instance, the pesticide paraquat has been widely used as a model for PD in different animals, as zebrafish and rodents [3], as it has been demonstrated that brain mitochondria are the major site of radical anion superoxide production following paraquat exposure [4]. In agreement, agents that improve mitochondrial function have been shown to exert beneficial effects in animal models and also in human patients [5–7].

In this context, organic compounds containing selenium have

* Corresponding author at: Universidade Federal do Pampa – UNIPAMPA, Programa de Pós-Graduação em Bioquímica, BR 472, Km 592, Caixa Postal 118, CEP 97500-970, Uruguaiana, RS, Brazil.
E-mail address: daianaavila@unipampa.edu.br (D.S. Ávila).

https://doi.org/10.1016/j.jtemb.2019.01.017
Received 25 August 2018; Received in revised form 12 January 2019; Accepted 30 January 2019

gained attention in the antioxidant therapy research and can complement natural cell defenses against prooxidant agents. Many of these molecules can form the nucleophile selenol (SeH), which can degrade peroxides and neutralize them [8,9]. The SeH presence, for instance, is essential to the reaction that occurs at the active site of five isoforms of glutathione peroxidase and three isoforms of thioredoxin reductase, two selenoenzymes, which has a higher reducing potential than thiol-containing isoforms [2,10,11]. Other molecules can form selenoxides after decomposing peroxides [12]. These molecules can also modulate the antioxidant redox system by activating transcriptional factors such as Nrf2 and FoxO, therefore increasing the expression of enzymatic antioxidants [13–17]. A good antioxidant must have one or more of these activities and also low levels of toxicity, which is often caused by thiol oxidation [8,18,19]. Notably, it has been reported that some organoselenium compounds as ebselen, diphenyl diselenide and 3′3-ditrifluoromethyldiphenyl diselenide have protected mitochondria from oxidative damage- induced by different prooxidant agents [20].

In order to screen new potential drugs that successfully treat oxidative stress- associated diseases, a model organism which is feasible for fast, low cost, and reproducible biological activity and toxicity evaluation is desirable. In addition, the model should allow translatability to mammal models. One of these models is the nematode *Caernorhabditis elegans*, basically because of its considerable genetic homology to more mammal species, particularly to humans [21]. Previously, our group described that two Se and Te-xylofuranosides modulated the cellular sublocation of FOXO/DAF-16 in *C. elegans*, thus protecting from Mn-induced toxicity [16]. This pathway was also essential for the recovery from oxidative damage induced by paraquat after 4-phenylchalcogenil-7-chloroquinolines, which also demonstrated to interact with the Nrf2/SKN-1 pathway, increasing the expression of gcs-1, sod-3 and gst-4, thus improving the stress response in *C. elegans* [15]. Notably, paraquat and Mn have induced mitochondrial damage by increasing ROS production [22,23].

Considering that synthetic organic Se compounds may represent novel therapeutic targets for the various diseases in which oxidative stress is involved, we sought to evaluate the biological activity of a series of organoselenotriazoles over the nematode *C. elegans*. Herein we demonstrated that these molecules have potential to repair oxidative damage caused by different mitochondrial stress inducers, particularly from a genetic mitochondrial dysfunction model (*mev-1* mutation).

2. Materials and methods

2.1. Compounds and chemicals

4-Phenyl-1-(phenylselanylmethyl)-1,2,3-triazole (Se-TZ) and their derivatives (Fig. 1) were synthesized according to Seus, Saraiva, Alberto, Savegnago and Alves [24] and were solubilized in DMSO. All other reagents were obtained from Sigma (St. Louis, MO, USA) or from local suppliers.

2.2. C. elegans strains and maintenance

C. elegans strains were routinely propagated at 20 °C on Nematode Growth Medium (NGM) plates containing a lawn of *Escherichia coli* strain OP50 as food source. The synchronization was carried out by treatment with sodium hypochlorite in hermaphrodites during the reproductive period, where eggs are isolated [25]. 14 h later, first larval staged worms (L1) hatched from the eggs and were then used in the experiments. The following strains were used for this study: N2 Bristol (wildtype) and TK22 [*mev-1*(*kn1*) III], which were obtained from Caenorhabditis Genetics Center (CGC, University of Minnesota, MN, USA). Both strains were used for the assays outlined below.

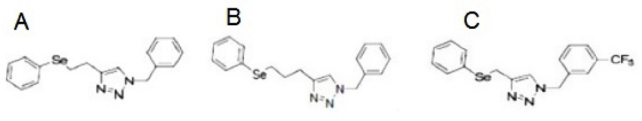

Fig. 1. Chemical Structure of the tested molecules: A) Se-Tz; B) Se-Tz Ia; C) Se-Tz Ib.

2.3. Determination of lethal concentration 50% (LC_{50})

Worms (1500) at the first larval stage (L1), previously synchronized as described above, were exposed to the compounds for 30 min in 0.5% NaCl without any food. Following treatment, they were washed three times with 0.5% NaCl solution to remove the compounds and transferred to NGM plates with *E. coli* OP50 as food source. We used concentrations ranging from 1 μM to 1000 μM to determine the LC_{50} based on the worms survival rate 48 h after the exposure. As DMSO was used as vehicle for the compounds at 0.5%, a control with the same concentration was used.

2.4. Lifespan assay

After the acute exposure to compounds, 20 live worms (in duplicates) at the same developmental stage were collected at the late L4 stage and transferred daily (during the reproductive period) to new plates NGM seeded with OP50 in order to prevent contamination or progeny. Worms were transferred every two days after reproductive period. Survival was assessed daily until all the subjects were dead and this experiment has been repeated at least 3 times.

2.5. Stress-resistance assays

2.5.1. Chemical stress

To evaluate the protective potential of the compounds against toxic agents we used the pesticide paraquat and the prooxidant hydrogen peroxide (H_2O_2). We exposed N2 worms at L1 stage to paraquat (Gramoxone 200®-1 mM) or H_2O_2 (0.5 mM) for 30 min, in a liquid medium containing 0.5% NaCl. Right after the washes to remove the toxicants, worms were exposed to the compounds (10 μM) as stated previously. After 30 min, treatments were washed off to remove the compounds and finally placed on plates with *E. coli* OP50. The survival and other parameters outlined in sequence have been assessed 48 h after exposures.

2.5.2. Genetic stress-model

For this type of stress we have used *mev-1* mutants, which present abnormal energy metabolism due to a mutation in this orthologous of succinate dehydrogenase cytochrome b560 subunit (complex II). Therefore, worms depict increased sensitivity to oxidative stress that results in shortened lifespan. Mutants were treated as previously described for wildtype. 48 h following exposure, 20 live animals (in duplicates) were transferred to new plates and lifespan was followed as previously described.

2.6. Determination of reactive oxygen species (ROS) in vivo

The intracellular levels of ROS were measured using 2′7′dicloroflurescein-diacetate (H_2DCF-DA) (Sigma) according to Shi, Yu, Liao and Pan [26] with modifications. 48 h after the treatments described above, worms at the fourth larval stage (L4) were washed three times with water to remove the bacteria then incubated with 50 μM of 2′7′dicloroflurescein-diacetate (H_2DCF-DA) for 1 h. Subsequently, they were washed with distilled water twice and transferred to 96-well microplate. Fluorescence levels were measured (excitation 485 nm and emission 535 mm) using a microplate reader (Spectramax M5 Molecular Devices). Fluorescence measurements were normalized by protein quantified according to Bradford [27].

2.7. SOD and catalase activities assays

5000 worms from both strains were treated with the compounds at 10 μM and allowed to reach the L4 stage. For samples preparation, animals were washed off plates with M9 buffer (0.02 M KH_2PO_4, 0.04 M Na_2HPO_4, 0.08 M NaCl, and 0.001 M $MgSO_4$) for bacteria removal and were subsequently frozen and thawed 3 times. Finally, samples were sonicated using a lysis buffer (10 mM tris − HCl pH 7.5, 150 mM NaCl and 0.1 mM EDTA), centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant was separated for the assays. Activity of the enzyme superoxide dismutase was measured according to Boveris [28], with some adaptations. Different volumes of supernatant were used for determination of epinephrine oxidation curve. To determine the activity of catalase, degradation of H_2O_2 was monitored as described by Aebi, 1984 [29]. Data were normalized for protein measurement according Bradford [27].

2.8. Determination of non-proteic thiol groups (NPSH) levels

The quantification of NPSH was conducted according to Ellman [30]. 48 h following treatment, worms were washed from the NGM plates with M9 buffer, frozen and thawed 3 times, sonicated and centrifuged for 10 min at 12,000 rpm (4 °C). After precipitating protein content with TCA 25%, DTNB (5,5′-Dithiobis-2-Nitrobenzoic Acid 2 mM) and Tris HCl (1 M) were added to the samples that were incubated for 5 min at room temperature. Samples were transferred to 96-well plates and along with the standard curve of L-cysteine were read at 412 nm in a microplate reader. The data were normalized by the amount of protein [27].

2.9. Statistical analysis

All experiments were repeated at least 3 independent times in duplicates. For survival, we performed a dose-response curve and plotted a sigmoidal dose response curve using nonlinear regression followed by a Bonferroni post hoc test. For longevity assays, the curves were plotted and repeated measures ANOVA was performed. For the other analysis one-way ANOVA followed by a Tukey post hoc test was performed. For all the experiments, the effects of the treatments were compared to untreated controls assayed in parallel. P values < 0.05 were considered statistically significant. In all figures, the standard error of the mean is depicted.

3. Results

3.1. Organoselenotriazoles have low toxicity

Initially we evaluated the survival of the worms against several organoselenotriazoles concentrations (1–1000 μM) to determine their toxicity. The concentrations here employed did not significantly induce mortality, then it was not possible to determine the lethal concentration 50% (LC_{50}) (Fig. 2). Based on previous studies of our group [15,16], the concentration of 10μM was chosen for the following tests. Fig. 3A shows that this concentration did not influence their survival and did not affect the formation of ROS determined by H_2DCF-DA fluorescence (Fig. 3B). We also found that worms lifespan (Fig. 3C) and the levels of non-proteic thiols (Fig. 3D) were not affected by any of the compounds at this non-lethal concentration.

3.2. Organoselenotriazoles partially reverse the damage induced by paraquat and H_2O_2

Paraquat (1 mM), a potent pro-oxidant agent capable of inducing oxidative stress through increased radical superoxide anion production, caused a significant reduction in worms survival upon exposure. Treatment with organoselenotriazoles partially rescued this effect

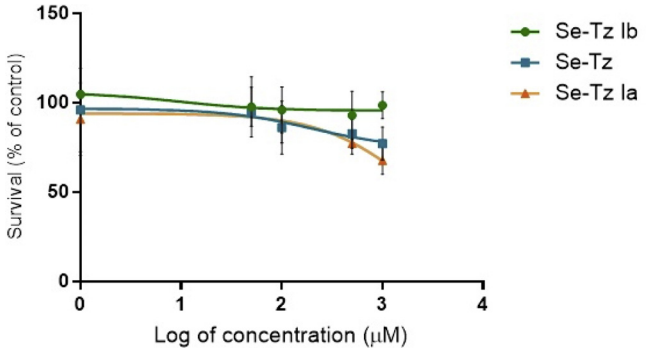

Fig. 2. Dose response curve of selenotriazoles in *C. elegans*. Concentrations are represented as log of concentration (μM) for better plotting.

(Fig. 4A). On the other hand, none of the molecules was able to reduce ROS formation induced by paraquat treatment (Fig. 4B).

To verify whether another source of stress would elicit distinct responses we have used H_2O_2 (0.5 mM), which significantly affected survival rate. However, in this condition only compound Se-Tz Ia partially rescued from H_2O_2- induced mortality (Fig. 5A). In addition, the compounds did not reduce the increased ROS formation following H_2O_2 exposure (Fig. 5B).

3.3. Organoselenotriazoles reduce ROS associated deleterious effects in a genetic model of oxidative stress

To observe whether animals would react against an endogenous source of ROS we used the *mev-1* knockout mutant. These worms have a reduced lifespan due to exacerbated production of ROS in mitochondria by incomplete reduction of O_2 in the electron transporter chain. The worms treated with the different selenotriazoles showed a significant increase in lifespan when compared to the control group. In addition, compounds Se-Tz Ia and Se-Tz Ib were able to increase the lifespan at the level of N2 (wildtype) nematodes, which were used as control strain (Fig. 6A). Additionally, the same compounds that depicted better effect on longevity (Se-Tz Ia and Se-Tz Ib), significantly reduced ROS production in *mev-1* mutants (Fig. 6B).

3.4. Organoselenotriazoles modulate catalase enzyme activity

We also assayed the activity of the enzymes superoxide dismutase and catalase and observed that *mev-1* mutants show an increase in CAT activity. The compounds significantly reduced the CAT activity in the mutants *mev-1* worms (Fig. 7B). However, SOD activity was not significantly altered (Fig. 7A). In addition, we have imaged SOD::GFP and we did not observe any alteration in this protein levels following treatment with these molecules (data not shown).

4. Discussion

Organic selenium compounds containing heterocycles in their structures have been highlighted in the literature for their wide pharmacological functions attributed mainly to their redox potential. In the entire perspective, this potential reflects in interesting effects as anticancer, hepatoprotective, neuroprotective and anti-depressant properties, just to name a few, for ebselen [31,32]. Based on the promising effects of three organoselenotriazoles we sought to verify their toxicity in worms and then investigate their potential in stress resistance against different mitochondrial stressors in *C. elegans*.

Initially, we have evaluated the effects of the compounds *per se* over wild-type animals in order to ascertain whether the compounds were toxic to them. According to the dose-response survival curve, the compounds showed no toxicity at the concentrations here tested

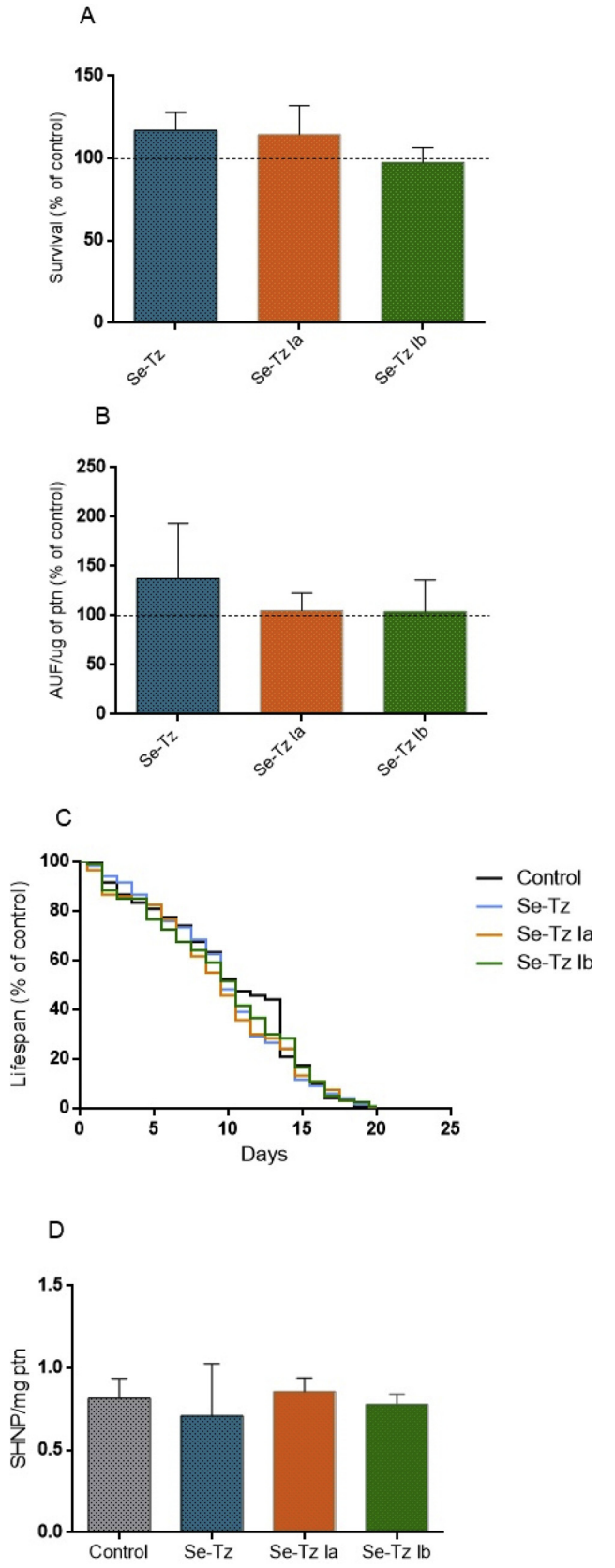

Fig. 3. *Per se* effect of organoselenotriazoles compounds A) non-lethal concentration of 10 μM on survival rate. Data were normalized to percentage of control B) ROS levels measured with H_2DCF-DA probe. Data were normalized to percentage of control C) Lifespan. D) Non-protein thiols. Data are expressed as mean ± SEM. * Indicates significant difference compared to the control group (dashed line).

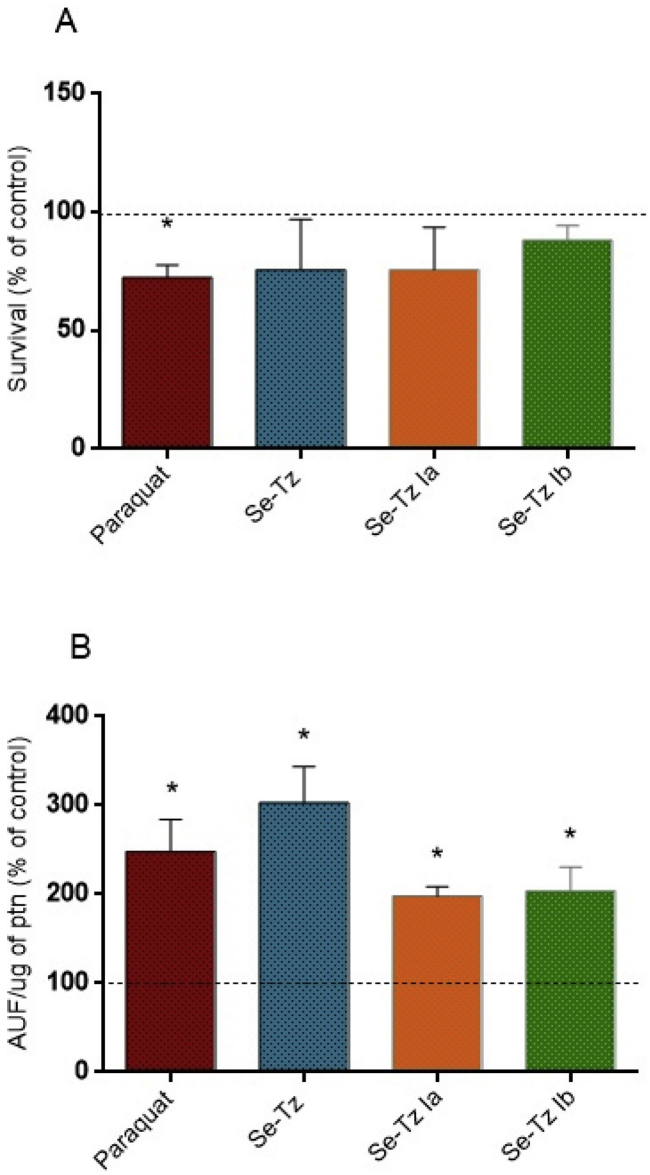

Fig. 4. Paraquat reversion protocol following organoselenotriazoles compounds treatments. A) Non-lethal concentration of 10 μM on survival, B) ROS levels measured with H_2DCF-DA probe. Data were normalized to percentage of control and expressed as mean ± SEM. * Indicates significant difference compared to the control group (dashed line).

(Fig. 1). This was a surprising result based on previous studies using the same experimental model, where different organochalcogen molecules had major differences between their LC_{50} values, but all depicted measurable toxicity [15,16].

We have proceeded with toxicity assessment through longevity and oxidative stress of worms acutely exposed to the non-lethal 10μM concentration of each compound (Fig. 3). The compounds did not affect any of the evaluated parameters *per se*, including ROS formation, which was determined using the fluorescent probe 2′7′-diclorofluorescein-diacetate (H_2DCF-DA). The molecular mechanism by which organic selenium compounds induce toxicity and oxidative stress is not well established, however, the interaction between selenium and thiols plays a central role in their molecular toxicity [19,33]. Indeed, thiol oxidation is known to contribute to the production of ROS, mediating toxicity by directly or indirectly damaging biomolecules [18,34]. In the present study, no alteration in non-protein thiols levels were observed following treatment with organoselenotriazoles (Fig. 3D), however

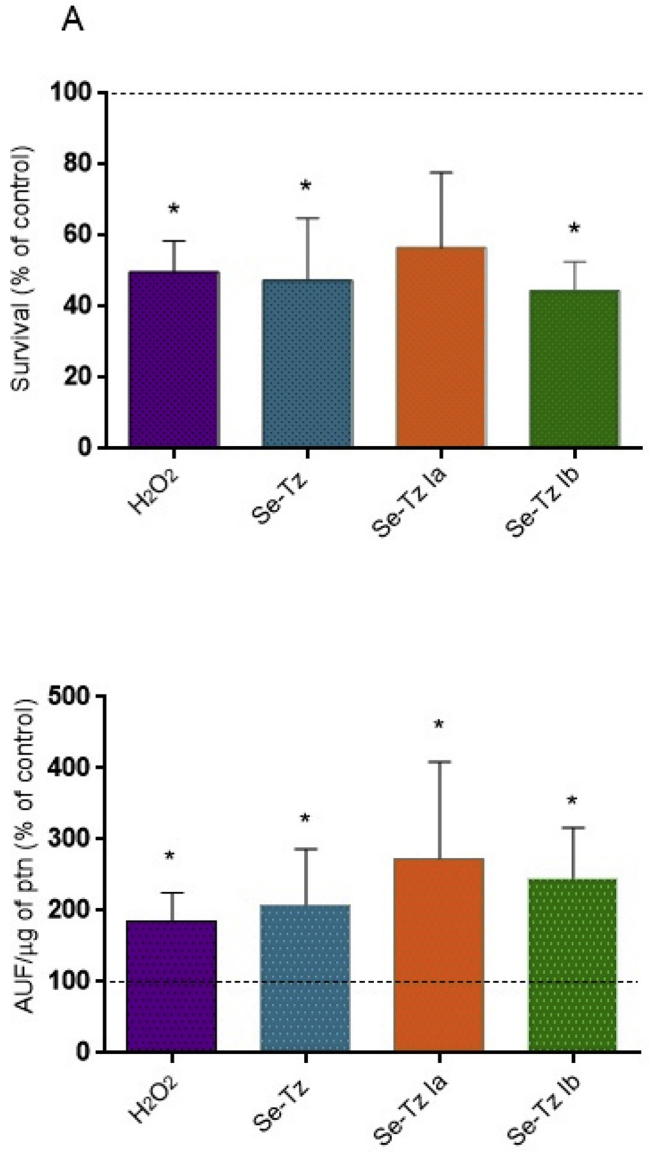

Fig. 5. H_2O_2 reversion protocol following organoselenotriazoles compounds treatment. A) non-lethal concentration of 10 µM on survival; B) ROS levels measured with H_2DCF-DA probe. Data were normalized to percentage of control and expressed as mean ± SEM. * Indicates significant difference compared to the control group (dashed line).

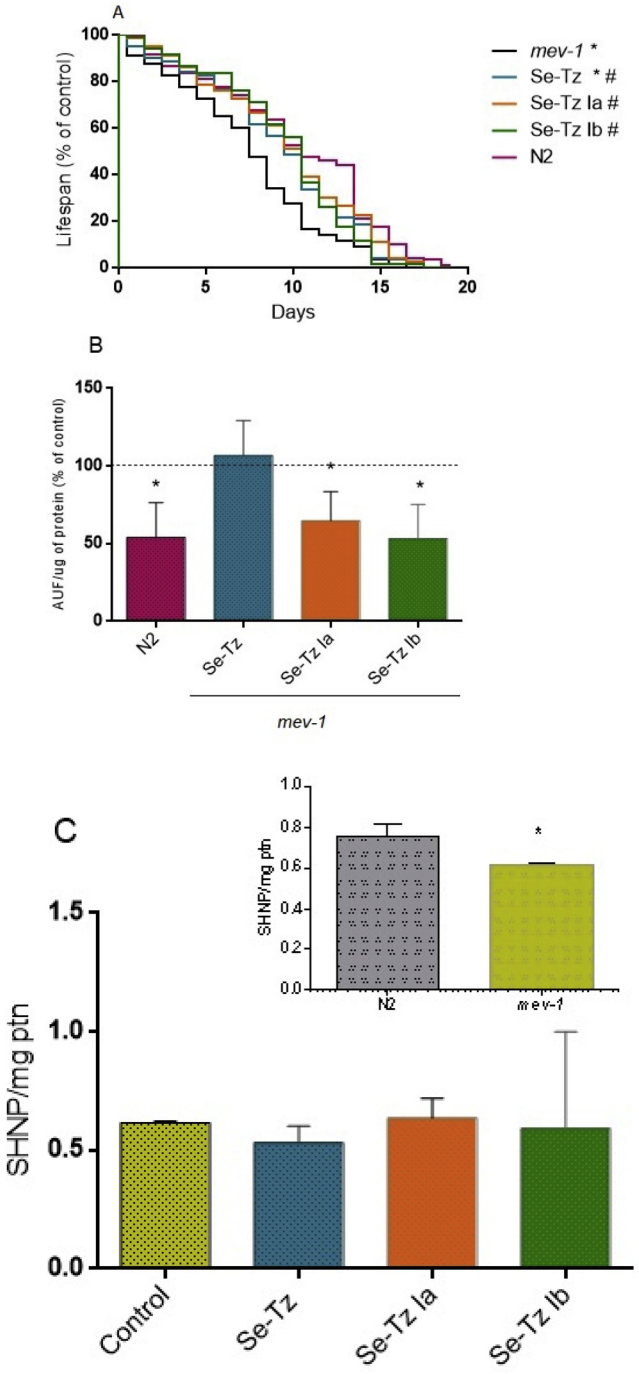

Fig. 6. *mev-1* mutants treated with organoselenotriazoles compounds. A) Lifespan; B) ROS levels measured with H_2DCF-DA probe; C) Non-protein thiols. Lifespan and ROS data were normalized to percentage of control. Data are expressed as mean ± SEM. * Indicates significant difference compared to the control group. # Indicates significant difference compared to the control N2 (wildtype).

Donato et al observed increased NPSH levels in liver and kidney of Se-Tz treated mice. This finding may be associated to the metabolization of organoselenium compounds. Studies have demonstrated that Se can be released from organic forms as aliphatic selenides, diphenyl diselenides and diphenyl selenides, thus providing free Se [35]. Considering the high catalytic activity of inorganic Se forms as selenite in oxidizing thiols and, particularly, GSH [36,37], we believe that the selenotriazoles did not release inorganic Se. For instance, a study of Wendel demonstrated that Se in Ebselen cannot be metabolized to the inorganic selenium pool [38].

Next, we sought to evaluate these molecules ability to repair the oxidative damage inflicted in *C. elegans* by two well-known prooxidant agents, paraquat and hydrogen peroxide. According to our results, organoselenotriazoles did not reduce ROS formation induced by pretreatment with neither paraquat nor hydrogen peroxide (Figs. 3B and 4 B). We also evaluated ROS levels immediately after treatment (L1) and no reduction was observed in animals exposed to organoselenotriazoles (data not shown). Notably, there was a partial rescue of this effect in the survival parameter, especially when the stressor agent was paraquat, a mitochondrial toxicant (Figs. 3A and 4 A). This may suggest that organoselenotriazoles may be more active against superoxide anions produced in the mitochondria by paraquat than against hydrogen peroxide. Indeed, it has been already shown that *C. elegans sod-3* may be upregulated after acute exposure to non-lethal concentrations of 4-phenylselenyl-7-chloroquinolines compounds, where CTL-2 (hydrogen peroxide detoxifying enzyme) involvement was not observed [15]. Also, there are literature records for organic selenium compounds that

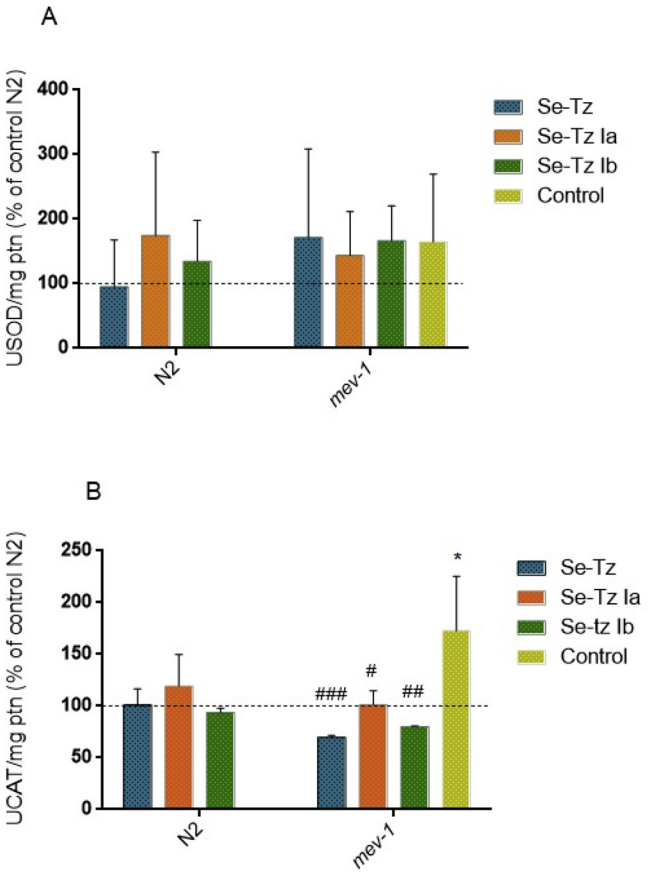

Fig. 7. Enzymes activities in wildtype and *mev-1* mutants following treatments with organoselenotriazoles compounds. A) Superoxide dismutase. B) Catalase. Data were normalized to percentage of control and expressed as mean ± SEM. * Indicates significant difference compared to the control N2 (wildtype) group. # Indicates significant difference compared to *mev-1*.

may mimic superoxide dismutase activity *in vitro* [39], however, the same has not been reported for catalase.

Hence, taking advantage of one of the main features of *C. elegans*, which is the easy availability of transgenic strains, we employed *mev-1* mutants. These animals have a dysfunction of succinate dehydrogenase enzyme of the mitochondrial respiratory chain and, consequently, have an abnormal energy metabolism with increased sensitivity to oxidative stress, resulting in higher ROS levels and elevated ROS-mediated damage contributing to shortened lifespan [40–42]. Indeed, we have observed reduced lifespan as well as reduced thiols groups in these mutants, which reassures their disturbed oxidative metabolism (Fig. 5C-insert). The organoselenotriazoles compounds were able to improve *mev-1* reduced lifespan when compared to the control group. In addition, Se-Tz Ia and Se-Tz Ib- treated worms depicted lifespans that were indistinguishable from wild-type, thus indicating that these compounds totally recovered the shortened lifespan induced by oxidative stress (Fig. 5A). This effect is possibly due to the reduction in ROS formation (Fig. 5B). As a consequence, the increased catalase activity in *mev-1* mutants was reduced by compounds treatment (Fig. 6B). Interestingly, in a previous study the catalase activity in Se-TZ treated mice was increased [43]. Hence we believe that the compound may act differently according to the stress condition, thus acting as scavenger at high levels of ROS, reducing the need for antioxidant enzymes (CAT and SOD). The increased CAT activity in *mev-1* mutants may be a compensatory response to the high production of ROS, which might be a major cause of their reduced lifespan, as it is broadly reported in the literature [44,45]. We suggest that the compounds neutralized ROS production and consequently decreased the need of antioxidant defenses, such as catalase. Increased lifespan may be linked to decreased oxidative stress in *C. elegans* [46] and other organisms [47]. As previously stated, selenotriazoles compounds may be more efficient against superoxide anions than against hydrogen peroxide, which would in turn explain why catalase activity seems to be decreased (Fig. 6A), once it would be not necessary in the absence of its precursor, the superoxide anion.

The interaction of organochalcogens with thiols of redox regulated proteins and enzymes leads to unexpected biological effects [8,19]. This thiol- peroxidase activity has been considered the major mechanism by which these molecules depict pharmacological properties, at the expenses of GSH and NADPH, thus generating the selenol intermediate that can react and neutralize ROS. In our study, we did not observe any depletion of NPSH levels (mostly GSH) by selenotriazoles treatment, however our group has demonstrated that 4-phenylselanyl-7-chloroquinoline (PSQ) activates γ-glutamyl cysteine synthase (γGCS) in worms, which is the rate-limiting enzyme in GSH synthesis. Therefore, putatively depleted GSH levels could be recovered from increased biosynthesis.

In addition, many studies have evidenced that organoselenium compounds can improve ROS-induced mitochondrial damage [48–51]. Ebselen can reduce cytochrome c release form mitochondria following an ischemic event in mice [52]. Fiuza et al., for instance, demonstrated that $(PhSe)_2$ treatment in BAEC cells increased the expression of peroxiredoxins, thus protecting mitochondria from the damage induced by peroxynitrite [53]. Furthermore, another study evidenced that $(PhSe)_2$ and ebselen can increase the expression of glutathione peroxidase (GPx) as a consequence of Nrf2 translocation in BAEC cells [13]. Our group has demonstrated in *C. elegans* that different organoselenium compounds modulate another transcriptional factor, DAF-16/FOXO [54,55], which modulates, amongst other proteins, peroxiredoxin-3 [56].

Selenotriazoles have demonstrated antidepressant-like effect mediated, at least partially, *via* the central dopaminergic and serotoninergic neurotransmitter systems [43]. Here we demonstrated that this class of compounds can protect against specific mitochondrial stressor, the *mev-1* mutation, and that the most putative mechanism is by scavenging activity, as the molecular targets SOD and CAT were not modified by these molecules. However, modulation of transcriptional factor and the expression of important antioxidants enzymes by these selenotriazoles cannot be ruled out.

Conflict of interest

Authors declare no conflict of interest in the execution of this study.

Acknowledgments

Authors thank CNPq (Universal Grants), FAPERGS (PqG # 18/2551-0000434-0), CNPq/FAPERGS/DECIT/SCTIE-MS/PRONEM #16/2551-0000248-7 and UNIPAMPA for their funding. A.T.G.S. received CAPES scholarship. D.S.A is recipient of researcher fellowship (PQ2/CNPq).

References

[1] A. Gaiz, S. Mosawy, N. Colson, I. Singh, Thrombotic and cardiovascular risks in type two diabetes: role of platelet hyperactivity, Biomed. Pharmacother. 94 (2017) 679–686.
[2] G.H. Kim, J.E. Kim, S.J. Rhie, S. Yoon, The role of oxidative stress in neurodegenerative diseases, Exp. Neurobiol. 24 (4) (2015) 325–340.
[3] R.J. Dinis-Oliveira, F. Remiao, H. Carmo, J.A. Duarte, A.S. Navarro, M.L. Bastos, F. Carvalho, Paraquat exposure as an etiological factor of Parkinson's disease, Neurotoxicology 27 (6) (2006) 1110–1122.
[4] P.R. Castello, D.A. Drechsel, M. Patel, Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain, J. Biol. Chem. 282 (19) (2007) 14186–14193.
[5] G. Fiskum, A. Starkov, B.M. Polster, C. Chinopoulos, Mitochondrial mechanisms of neural cell death and neuroprotective interventions in Parkinson's disease, Ann. N. Y. Acad. Sci. 991 (2003) 111–119.
[6] A. Storch, Coenzyme Q10 in Parkinson's disease. Symptomatic or neuroprotective

effects? Nervenarzt 78 (12) (2007) 1378–1382.
[7] A. Storch, W.H. Jost, P. Vieregge, J. Spiegel, W. Greulich, J. Durner, T. Muller, A. Kupsch, H. Henningsen, W.H. Oertel, G. Fuchs, W. Kuhn, P. Niklowitz, R. Koch, B. Herting, H. Reichmann, Randomized, double-blind, placebo-controlled trial on symptomatic effects of coenzyme Q(10) in Parkinson disease, Arch. Neurol. 64 (7) (2007) 938–944.
[8] N.V. Barbosa, C.W. Nogueira, P.A. Nogara, A.F. de Bem, M. Aschner, J.B.T. Rocha, Organoselenium compounds as mimics of selenoproteins and thiol modifier agents, Metallomics 9 (12) (2017) 1703–1734.
[9] J.B.T. Rocha, B.C. Piccoli, C.S. Oliveira, Biological and chemical interest in selenium: a brief historical account, Arkivoc 2 (2017) 457–491.
[10] C. Jacob, G.I. Giles, N.M. Giles, H. Sies, Sulfur and selenium: the role of oxidation state in protein structure and function, Angew. Chem. Int. Ed. Engl. 42 (39) (2003) 4742–4758.
[11] H.J. Reich, R.J. Hondal, Why nature chose selenium, ACS Chem. Biol. 11 (4) (2016) 821–841.
[12] V. Nascimento, E.E. Alberto, D.W. Tondo, D. Dambrowski, M.R. Detty, F. Nome, A.L. Braga, GPx-like activity of selenides and selenoxides: experimental evidence for the involvement of hydroxy perhydroxy selenane as the active species, J. Am. Chem. Soc. 134 (1) (2012) 138–141.
[13] A.F. de Bem, B. Fiuza, P. Calcerrada, P.M. Brito, G. Peluffo, T.C. Dinis, M. Trujillo, J.B. Rocha, R. Radi, L.M. Almeida, Protective effect of diphenyl diselenide against peroxynitrite-mediated endothelial cell death: a comparison with ebselen, Nitric Oxide 31 (2013) 20–30.
[14] G. Roseni Mundstock Dias, R. Medeiros Golombieski, R. de Lima Portella, G. Pires do Amaral, F. Antunes Soares, J.B. Teixeira da Rocha, C. Wayne Nogueira, N. Vargas Barbosa, Diphenyl diselenide modulates gene expression of antioxidant enzymes in the cerebral cortex, hippocampus and striatum of female hypothyroid rats, Neuroendocrinology 100 (1) (2014) 45–59.
[15] W.G. Salgueiro, B.S. Goldani, T.V. Peres, A. Miranda-Vizuete, M. Aschner, J.B.T. da Rocha, D. Alves, D.S. Avila, Insights into the differential toxicological and antioxidant effects of 4-phenylchalcogenil-7-chloroquinolines in Caenorhabditis elegans, Free Radic. Biol. Med. 110 (2017) 133–141.
[16] S.G.N. Wollenhaupt, A.T. Soares, W.G. Salgueiro, S. Noremberg, G. Reis, C. Viana, P. Gubert, F.A. Soares, R.F. Affeldt, D.S. Lüdtke, F.W. Santos, C.C. Denardin, M. Aschner, D.S. Avila, Seleno- and telluro-xylofuranosides attenuate Mn-induced toxicity in C. elegans via the DAF-16/FOXO pathway, Food Chem. Toxicol. 64 (2014) 192–199.
[17] D.S. Avila, A. Benedetto, C. Au, F. Manarin, K. Erikson, F.A. Soares, J.B. Rocha, M. Aschner, Organotellurium and organoselenium compounds attenuate Mn-induced toxicity in Caenorhabditis elegans by preventing oxidative stress, Free Radic. Biol. Med. 52 (9) (2012) 1903–1910.
[18] C.W. Nogueira, J.B. Rocha, Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds, Arch. Toxicol. 85 (11) (2011) 1313–1359.
[19] L. Orian, S. Toppo, Organochalcogen peroxidase mimetics as potential drugs: a long story of a promise still unfulfilled, Free Radic. Biol. Med. 66 (2014) 65–74.
[20] R.L. Puntel, D. Avila, D.H. Roos, S. Pinton, Mitochondrial Effects of Organoselenium and Organotellurium Compounds, (2015).
[21] C.F. Labuschagne, A.B. Brenkman, Current methods in quantifying ROS and oxidative damage in Caenorhabditis elegans and other model organism of aging, Ageing Res. Rev. 12 (4) (2013) 918–930.
[22] I. Abdulwahid Arif, H. Ahmad Khan, Environmental toxins and Parkinson's disease: putative roles of impaired electron transport chain and oxidative stress, Toxicol. Ind. Health 26 (2) (2010) 121–128.
[23] D.S. Avila, D. Colle, P. Gubert, A.S. Palma, G. Puntel, F. Manarin, S. Noremberg, P.C. Nascimento, M. Aschner, J.B. Rocha, F.A. Soares, A possible neuroprotective action of a vinylic telluride against Mn-induced neurotoxicity, Toxicol. Sci. 115 (1) (2010) 194–201.
[24] N. Seus, M.T. Saraiva, E.E. Alberto, L. Savegnago, D. Alves, Selenium compounds in click chemistry: copper catalyzed 1,3-dipolar cycloaddition of azidomethyl arylselenides and alkynes, Tetrahedron 68 (51) (2012) 10419–10425.
[25] S. Brenner, The genetics of Caenorhabditis elegans, Genetics 77 (1) (1974) 71–94.
[26] Y.C. Shi, C.W. Yu, V.H. Liao, T.M. Pan, Monascus-fermented dioscorea enhances oxidative stress resistance via DAF-16/FOXO in Caenorhabditis elegans, PLoS One 7 (6) (2012) e39515.
[27] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[28] A. Boveris, Determination of the production of superoxide radicals and hydrogen peroxide in mitochondria, Meth. Enzymol. 105 (1984) 429–435.
[29] H. Aebi, Catalase in vitro, Meth. Enzymol. 105 (1984) 121–126.
[30] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1) (1959) 70–77.
[31] T. Posser, M.P. Kaster, S.C. Barauna, J.B. Rocha, A.L. Rodrigues, R.B. Leal, Antidepressant-like effect of the organoselenium compound ebselen in mice: evidence for the involvement of the monoaminergic system, Eur. J. Pharmacol. 602 (1) (2009) 85–91.
[32] T. Satoh, K. Ishige, Y. Sagara, Protective effects on neuronal cells of mouse afforded by ebselen against oxidative stress at multiple steps, Neurosci. Lett. 371 (1) (2004) 1–5.
[33] D. Grey, Tellurium: Properties, Uses, and Research, (2017).
[34] B. Jernstrom, R. Morgenstern, P. Moldeus, Protective role of glutathione, thiols, and analogues in mutagenesis and carcinogenesis, Basic Life Sci. 61 (1993) 137–147.
[35] W.J. Adams Jr., J.J. Kocsis, R. Snyder, Acute toxicity and urinary excretion of diphenyldiselenide, Toxicol. Lett. 48 (3) (1989) 301–310.
[36] C.C. Tsen, A.L. Tappel, Catalytic oxidation of glutathione and other sulfhydryl compounds by selenite, J. Biol. Chem. 233 (5) (1958) 1230–1232.
[37] J. Rocha, B. Piccoli, C. Oliveira, Biological and chemical interest in selenium: a brief historical account, Arch. Org. Chem. 2 (2017) 457–491.
[38] A. Wendel, M. Fausel, H. Safayhi, G. Tiegs, R. Otter, A novel biologically active seleno-organic compound–II. Activity of PZ 51 in relation to glutathione peroxidase, Biochem. Pharmacol. 33 (20) (1984) 3241–3245.
[39] C.W. Nogueira, G. Zeni, J.B. Rocha, Organoselenium and organotellurium compounds: toxicology and pharmacology, Chem. Rev. 104 (12) (2004) 6255–6285.
[40] C.W. Yu, C.C. Wei, V.H. Liao, Curcumin-mediated oxidative stress resistance in Caenorhabditis elegans is modulated by age-1, akt-1, pdk-1, osr-1, unc-43, sek-1, skn-1, sir-2.1, and mev-1, Free Radic. Res. 48 (3) (2014) 371–379.
[41] S. Fong, L.F. Ng, L.T. Ng, P.K. Moore, B. Halliwell, J. Gruber, Identification of a previously undetected metabolic defect in the complex II Caenorhabditis elegans mev-1 mutant strain using respiratory control analysis, Biogerontology 18 (2) (2017) 189–200.
[42] T. Ishii, M. Miyazawa, P.S. Hartman, N. Ishii, Mitochondrial superoxide anion (O(2)(-)) inducible "mev-1" animal models for aging research, BMB Rep. 44 (5) (2011) 298–305.
[43] F. Donato, M.G. de Gomes, A.T. Goes, N. Seus, D. Alves, C.R. Jesse, L. Savegnago, Involvement of the dopaminergic and serotonergic systems in the antidepressant-like effect caused by 4-phenyl-1-(phenylselanylmethyl)-1,2,3-triazole, Life Sci. 93 (9–11) (2013) 393–400.
[44] C.E. Schaar, D.J. Dues, K.K. Spielbauer, E. Machiela, J.F. Cooper, M. Senchuk, S. Hekimi, J.M. Van Raamsdonk, Mitochondrial and cytoplasmic ROS have opposing effects on lifespan, PLoS Genet. 11 (2) (2015) e1004972.
[45] B.M. Dancy, M.M. Sedensky, P.G. Morgan, Effects of the mitochondrial respiratory chain on longevity in C. elegans, Exp. Gerontol. 56 (2014) 245–255.
[46] Y. Honda, M. Tanaka, S. Honda, Redox regulation, gene expression and longevity, Geriatr. Gerontol. Int. 10 (Suppl. 1) (2010) S59–69.
[47] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, Nature 408 (6809) (2000) 239–247.
[48] G. Mancini, M. Raniel Straliotto, J.B. da Rocha, A.F. de Bem, Diphenyl diselenide improves the antioxidant response via activation of the Nrf-2 pathway in macrophage cells, Free Radic. Biol. Med. 75 (Suppl. 1) (2014) S40.
[49] C.B. Quines, P.M. Chagas, D. Hartmann, N.R. Carvalho, F.A. Soares, C.W. Nogueira, (p-ClPhSe)2 reduces hepatotoxicity induced by monosodium glutamate by improving mitochondrial function in rats, J. Cell. Biochem. 118 (9) (2017) 2877–2886.
[50] M.A. Hort, M.R. Straliotto, J. de Oliveira, N.D. Amoedo, J.B. da Rocha, A. Galina, R.M. Ribeiro-do-Valle, A.F. de Bem, Diphenyl diselenide protects endothelial cells against oxidized low density lipoprotein-induced injury: involvement of mitochondrial function, Biochimie 105 (2014) 172–181.
[51] J. de Oliveira, E.L. Moreira, G. Mancini, M.A. Hort, A. Latini, R.M. Ribeiro-do-Valle, M. Farina, J.B. da Rocha, A.F. de Bem, Diphenyl diselenide prevents cortico-cerebral mitochondrial dysfunction and oxidative stress induced by hypercholesterolemia in LDL receptor knockout mice, Neurochem. Res. 38 (10) (2013) 2028–2036.
[52] S. Namura, I. Nagata, S. Takami, H. Masayasu, H. Kikuchi, Ebselen reduces cytochrome c release from mitochondria and subsequent DNA fragmentation after transient focal cerebral ischemia in mice, Stroke 32 (8) (2001) 1906–1911.
[53] B. Fiuza, N. Subelzu, P. Calcerrada, M.R. Straliotto, L. Piacenza, A. Cassina, J.B. Rocha, R. Radi, A.F. de Bem, G. Peluffo, Impact of SIN-1-derived peroxynitrite flux on endothelial cell redox homeostasis and bioenergetics: protective role of diphenyl diselenide via induction of peroxiredoxins, Free Radic. Res. 49 (2) (2015) 122–132.
[54] W.G. Salgueiro, M.C. Xavier, L.F. Duarte, D.F. Camara, D.A. Fagundez, A.T. Soares, G. Perin, D. Alves, D.S. Avila, Direct synthesis of 4-organylsulfenyl-7-chloro quinolines and their toxicological and pharmacological activities in Caenorhabditis elegans, Eur. J. Med. Chem. 75 (2014) 448–459.
[55] S.G. Wollenhaupt, A.T. Soares, W.G. Salgueiro, S. Noremberg, G. Reis, C. Viana, P. Gubert, F.A. Soares, R.F. Affeldt, D.S. Ludtke, F.W. Santos, C.C. Denardin, M. Aschner, D.S. Avila, Seleno- and telluro-xylofuranosides attenuate Mn-induced toxicity in C. elegans via the DAF-16/FOXO pathway, Food Chem. Toxicol. 64 (2014) 192–199.
[56] X. Sun, W.D. Chen, Y.D. Wang, DAF-16/FOXO transcription factor in aging and longevity, Front. Pharmacol. 8 (2017) 548.