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# Diphenyl diselenide supplemented diet reduces depressive-like behavior in hypothyroid female rats

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## HIGHLIGHTS

- Hypothyroidism induced by methimazole triggered a depressive-like behavior in rats.
- Hypothyroidism also elicited cerebral stress oxidative responses.
- Dietary diphenyl diselenide was able to counteract these effects of hypothyroidism.
- Hypothyroidism is an interesting model to investigate depressive-like behavior.
- Diphenyl diselenide might be a promising compound to be tested under hypothyroidism.

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## ABSTRACT

Hypothyroidism has been associated to psychiatric disorder development and tissue oxidative damage. In this study, we evaluated the effect of diphenyl diselenide supplementation on depressive-like behavior triggered by methimazole exposure in female rats. Additionally, thiobarbituric acid reactive substances (TBARS), reactive oxygen species (ROS) and non-protein thiol (NP-SH) levels were analyzed in cerebral cortex, hippocampus and striatum structures of rats. Monoamine oxidase (MAO) activity was evaluated in total brain. Firstly, female rats received methimazole (MTZ) 20 mg/100 ml in the drinking water for 30 days and were evaluated in open-field and forced swimming tests (FST). In this set of experiments, the rats exposed to MTZ presented a depressive-like behavior, which was evidenced by a significant increase in the immobility time when compared to control group. Thereafter, MTZ-induced hypothyroid rats received either a standard or a diet containing 5 ppm of diphenyl diselenide, and then they were evaluated monthly in open-field and FST tests during 3 months. No alteration on the locomotor performance was observed among the groups. The depressive-like behavior of hypothyroid rats was blunted by diphenyl diselenide supplementation during all experimental periods. The levels of thyroid hormones remained low in MTZ exposed groups until the end of experimental period. The MTZ group had an increase in TBARS and ROS levels that were restored by diphenyl diselenide supplementation. NP-SH content of cerebral structures was not modified by MTZ exposure and/or diphenyl diselenide supplementation. Diphenyl diselenide supplementation restored the MAO B activity that was decreased in MTZ group. In summary, our results show that hypothyroidism induced by MTZ methimazole triggers a depressive-like behavior in female rats and that dietary diphenyl diselenide was able to reduce this effect.

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## 1. Introduction

Depression is a common and chronic illness characterized by some neurological symptoms such as depressed mood, anhedonia, irritability, difficulties in concentrating, and abnormalities in appetite and sleep [1]. In addition, depressed patients can present high rates of suicide behavior and are more susceptible in developing coronary artery disease and type 2 diabetes [2]. It is well established that the incidence, prevalence and morbidity risk of depression are greater in women than in men. Although, the underlying mechanisms of these gender differences are relatively unknown, there is evidence that the contribution of specific genes and gonadal hormones to depression-like behavior occurrence may differ between males and females [3–5,8]. Unfortunately the official diagnosis of depression is subjective and usually based on a documentation of a certain number of symptoms, which difficult the diagnosis and knowledge of action mechanisms of disease [1,6].

Evidence from literature demonstrates that thyroid dysfunction affects mainly women and may be linked to psychiatric disorders

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including depression [7–10]. The mechanisms by which thyroid hormones increase the risk for depression development remain to be established. Interestingly, it has been shown that thyroid hormones accelerate and improve the therapeutic response to tricyclic antidepressants and selective serotonin reuptake inhibitors in depressive patients [11,12]. Moreover, thyroid hormone treatment improves antidepressant pharmacotherapy even in patients with euthyroid hormonal state [13].

Hypothyroidism is a clinical condition characterized by elevated levels of the thyroid stimulating hormone (TSH) or by low levels of triiodothyronine (T3) and thyroxin (T4). The role of thyroid hormones on the development and functions of the central nervous system is well established [14,15]. The lack of thyroid hormones affects neuronal differentiation, migration, myelination, synaptogenesis, dendritic branching and plasticity processes [16–18]. Considering the impact of thyroid disease in people's health, the American Thyroid Association recommends that adults be screened for thyroid dysfunction by measurement of the serum TSH concentration, beginning at age 35 years and every 5 years thereafter [19,20].

Regarding therapeutic strategies on experimental depression, recently it has been demonstrated that diphenyl diselenide, a simple organoselenium compound, exhibits potent anxiolytic and antidepressant-like effects, which are related to central monoaminergic and serotoninergic systems and L-arginine/NO/cGMP pathway modulation [21–23]. In analogy, the antidepressant-like action of diphenyl diselenide was proven in malathion-exposed rats [24]. Indeed, diphenyl diselenide exhibits antioxidant and neuroprotective properties, which are closely related to its efficient mimetic activities of the enzymes glutathione peroxidase and thioredoxin reductase [25–29]. Taken together, the pharmacological properties of diphenyl diselenide could be interesting to verify under hypothyroidism condition, since it is linked to oxidative stress and neurological disturbances like cognitive deficits and depressive behavior [30–39]. Besides, our group recently demonstrated that the intake of diets supplemented with diphenyl diselenide improves spatial learning and memory deficits in hypothyroid rats [40].

In view of these considerations, this study was designed to examine whether the depressive-like behavior induced by methimazole exposure in adult female rats could be attenuated by dietary diphenyl diselenide. Additionally, we investigated the activity of monoamine oxidase (MAO) (E.C. 1.4.3.4) and some parameters of oxidative stress in the cerebral cortex, hippocampus and striatum of rats.

## 2. Materials and methods

### 2.1. Animals and reagents

Forty-eight adult female Wistar rats (≈200 g) purchased from our own breeding colony were acclimated for 10 days before the beginning of the experiments. The animals were housed in plastic cages and maintained at 22–24°°C, on a 12 h light/12 h dark cycle, with free access to food (Supra®, Brazil) and water. All experiments were performed in accordance with guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Santa Maria, RS, Brazil. Methimazole was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Diphenyl diselenide (Fig. 1) was synthesized according to literature methods [41]. Analysis of the $^{1}H$ NMR and $^{13}C$ NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. All other reagents were of analytical grade and obtained from standard commercial suppliers.

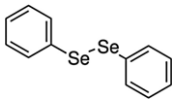



**Fig. 1.** Diphenyl diselenide structure.

### 2.2. Experimental protocol

#### 2.2.1. Induction of hypothyroidism

Hypothyroidism was induced by the administration of methimazole (MTZ) 0.02 g/100 ml, in the drinking water, as previously described by Dias et al. [40]. Initially, female rats were separated into two experimental groups (n = 48): control group, which received water and commercial food, and MTZ group, which received the solution of MTZ and commercial food. MTZ exposure via drinking water was maintained for 30 days and, after this period both groups were evaluated in open-field and forced swimming tests.

#### 2.2.2. Diphenyl diselenide supplementation

7 days after the experimental testing cited above, the female rats were randomly re-divided into 4 groups (n = 12): (1) control; (2) methimazole (MTZ); (3) diphenyl diselenide (Se) and (4) diphenyl diselenide plus methimazole (Se + MTZ). Control and MTZ groups were supplied with a standard diet, whereas Se and Se + MTZ groups received a diet supplemented with diphenyl diselenide at 5 ppm, which provided approximately 5 μg selenium/g of diet/per day (≈0.3 mg/kg body weight). The choice of this concentration was based in previous studies from our research group indicating that chronic dietary diphenyl diselenide did not cause toxic effects in rats [40,42,43]. The food was prepared in an industrial mixer to allow the uniformity of the mixture, and the diphenyl diselenide was dissolved in soybean oil. After preparation, the diets were frozen (4 °C) and offered ad libitum to the animals. The treatments were maintained concomitantly for three months. The behavioral assessments were performed monthly.

### 2.3. Behavioral testing

Open-field and forced swimming tests were performed monthly and conducted between 8:00 a.m. and 2:00 p.m.

#### 2.3.1. Open-field test

Locomotor and exploratory activities were evaluated by open-field test before forced swimming test. This task was performed in a circular apparatus (56 cm diameter) with the surface divided into 10 areas of equal size. The rats were gently placed at the center of the apparatus and observed for 5 min to record the locomotor (number of segments crossed) and exploratory activities (expressed by the number of time rearing on the hind limbs). The apparatus was cleaned between assessments with a 20% ethanol solution.

#### 2.3.2. Forced swimming test (FST)

FST was conducted using the method of Porsolt et al. [44]. Test was carried out over 2 days; where the first day was designed for pre-training and the second day for test session. Briefly, in the pre-training session the rats were individually placed for 15 min in open cylinders (45 cm height × 20 cm diameter) containing water, maintained at 25 ± 1 °C. The rats were then dried and heated by a lamp and returned to their cages. Twenty-four hours later, rats were placed again in the apparatus and the duration of immobility was recorded for 5 min. Each rat was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water.

### 2.4. Tissue preparation

At the end of the experimental period, the animals were euthanized and the whole blood collected by cardiac puncture for the measurement of total thyroid hormone (tT3 and tT4) levels. Heparinized blood was centrifuged at 3500 rpm for 5 min.

Brain was quickly removed, placed on ice and some dissected in the hippocampus, striatum and cerebral cortex. Cerebral structures and total brain were homogenized in cold 10 mM Tris–HCl pH 7.4. Homogenates

were centrifuged at 3500 rpm for 10 min to yield the low-speed supernatant fractions (S1) for lipid peroxidation, reactive oxygen species (ROS) and non-protein thiol (NP-SH) level determination. Monoamine oxidase (MAO) activity was assayed in mitochondrial preparation from total brain obtained by differential centrifugation [24]. Protein concentration was measured by the method of Bradford, using bovine serum albumin as the standard [45].

### 2.5. Thyroid hormones determination

Plasma levels of total T4 and total T3 were measured by microparticle enzyme immunoassay (MEIA) using AxSYM® system (Abbott Laboratories, Abbott Park, Illinois, USA), according to supplier's instructions.

### 2.6. Lipid peroxidation, ROS and NP-SH determination

Lipid peroxidation was performed by measuring thiobarbituric acid reactive substance (TBARS) levels, according to the method of Ohkawa et al. [46]. Briefly, aliquots of the supernatant fractions from cerebral structures were incubated at 100 °C for 1 h in acid medium containing 200 μl of 8.1% sodium dodecyl sulfate, 500 μl of 500 mM acetic acid buffer pH 3.4 and 500 μl of 0.6% thiobarbituric acid (TBA). TBARS levels were measured at 532 nm and the absorbance was compared with the standard curve using malondialdehyde (MDA). Results were expressed as nmol malondialdehyde/mg of protein.

ROS determination was based on the deacetylation of the 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) and its subsequent oxidation by intracellular reactive species to DCF, a fluorescent compound [47]. The supernatant fractions (S1) from cerebral structures were added to a medium with 10 mM Tris–HCl pH 7.4 and 1 mM DCFH-DA. Incubation was performed for 1 h in the dark until fluorescence measurement. DCF fluorescence intensity emission was recorded at 520 nm with 480 nm excitation. Results were expressed as μmol DCF/mg of protein, using a standard curve with DCF.

NP-SH levels were determined in the cerebral structures of rats by the method of Ellman [48]. Aliquots from S1 were mixed (1:1) with 10% trichloroacetic acid (TCA) and centrifuged at 3500 rpm for 10 min. After centrifugation, an aliquot of supernatant (200 μl) was added to a reaction medium containing 0.5 mM phosphate buffer pH 7.4, and 10 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB). NP-SH levels were measured at 412 nm, using a standard curve with reduced glutathione (GSH). Results were expressed as nmol SH/mg of protein.

### 2.7. Monoamine oxidase (MAO) activities

MAO activity was determined in total brain as described by Krajl [49] with modifications of Matsumoto et al. [50]. Aliquots of samples were incubated at 37 °C for 5 min in a medium containing $Na_2HPO_4$/$KH_2PO_4$ buffer pH 7.4 and specific inhibitors, selegiline (MAO B inhibitor, 250 ηM) or clorgiline (MAO A inhibitor, 250 ηM), at a final volume of 700 μl. Then kynuramine dihydrobromide was added to the reaction mixture (final concentration, 90 μM to MAO-A and MAO-T assays and 60 μM to MAO-B assay) as substrate. Samples were incubated at 37 °C for 30 min and reaction was stopped by adding trichloroacetic acid 10%. After centrifugation at 3000 ×g for 15 min, an aliquot of supernatant was added to 1 ml 1 M NaOH. The fluorescence intensity was detected with excitation at 315 nm and emission at 380 nm using a fluorescence spectrometer. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve. MAO activity was expressed as nmol of 4-hydroxyquinoline/mg of protein.

**Table 1**
Plasma tT3 and tT4 levels.

| | Control | MTZ | Se | MTZ + Se |
|---|---|---|---|---|
| tT3 | 0.68 ± 0.05 | 0.29 ± 0.03* | 0.58 ± 0.05 | 0.48 ± 0.03* |
| tT4 | 3.01 ± 0.25 | 0.53 ± 0.17* | 1.90 ± 0.39* | 1.47 ± 0.20* |

tT3 is expressed as ng/ml plasma and tT4 as pg/ml plasma. Values are expressed as means ± S.E.M. (5–8 rats/group).
* Denoted $p < 0.05$ from control group (two-way ANOVA followed Duncan's Test).

### 2.8. Statistical analysis

Data were analyzed by unpaired *T* Test and two-way ANOVA followed by Duncan's Multiple Range Test when appropriated. Data are presented as mean ± S.E.M. Differences among the groups were considered significant when $p < 0.05$.

## 3. Results

### 3.1. Thyroid hormones determination

Two-way ANOVA followed by Post Hoc comparisons showed that MTZ exposure was associated with low tT3 and tT4 plasma levels, which were not modified by dietary diphenyl diselenide treatment ($p > 0.05$) (Table 1). tT4 levels were lower in the group treated with diphenyl diselenide alone when compared to the control ($p > 0.05$).

Statistical analysis also revealed that there was a significant MTZ × Se interaction in both tT3 [$F(1,21) = 8.38$, $p < 0.008$] and tT4 [$F(1,20) = 11.27$, $p < 0.0031$] levels. Indeed, a main effect of Se was verified on the tT3 [$F(1,21) = 25.20$, $p < 0.0001$] and tT4 [$F(1,20) = 39.11$, $p = 0.0002$] content.

### 3.2. Open-field task and forced swimming test evaluation

No significant statistical difference was observed between control and MTZ groups in the crossing (unpaired *T* Test, $p = 0.73$) and rearing (unpaired *T* Test, $p = 0.501$) numbers before dietary diphenyl diselenide treatment. These data exclude the motor impairments that could influence the behavior of animals in the forced swimming test (Table 2).

Two-way ANOVA followed by Post Hoc comparisons revealed that there was no significant difference among the groups in the locomotor and exploratory activities evaluated 1, 2 and 3 months after dietary diphenyl diselenide treatment (Table 2). No significant main effect of

**Table 2**
Effects of MTZ and diphenyl diselenide supplementation on locomotor and exploratory activities in the open-field test.

| Hypothyroidism induction | | Groups | | |
|---|---|---|---|---|
| Parameters | | Control | | MTZ |
| Crossings | | 46.1 ± 2.47 | | 44.8 ± 2.86 |
| Rearing | | 14.5 ± 1.39 | | 16.0 ± 1.85 |
| Parameters | Control | MTZ | Se | MTZ + Se |
| *First month of treatment* | | | | |
| Crossings | 43.0 ± 3.88 | 44.1 ± 4.28 | 47.3 ± 4.02 | 38.5 ± 2.62 |
| Rearing | 19.6 ± 2.1 | 24.2 ± 3.4 | 18.3 ± 1.85 | 20.3 ± 2.14 |
| *Second month of treatment* | | | | |
| Crossings | 45.9 ± 3.43 | 35.1 ± 3.07 | 41.4 ± 3.6 | 38.2 ± 1.77 |
| Rearing | 13.4 ± 1.69 | 9.33 ± 1.87 | 8.0 ± 1.36 | 8.78 ± 1.08 |
| *Third month of treatment* | | | | |
| Crossings | 29.8 ± 2.71 | 24.8 ± 3.38 | 35.6 ± 2.38 | 28.8 ± 4.69 |
| Rearing | 11.6 ± 1.67 | 10.56 ± 1.38 | 14.3 ± 1.92 | 10.2 ± 2.98 |

Values are expressed as means ± S.E.M of 9–12 rats/group. Crossing and rearing are expressed as number of times (unpaired *T* Test/two-way ANOVA followed Duncan's Test).

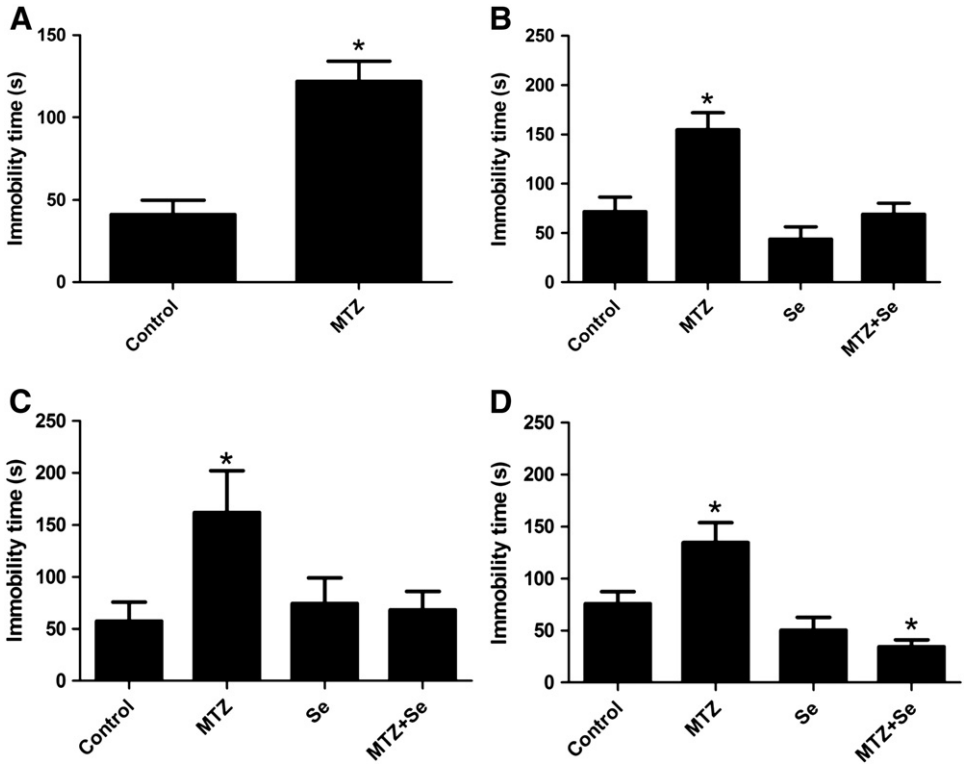



**Fig. 2.** Effects of MTZ and diphenyl diselenide supplementation on the immobility time in the FST. (A): FST in the period of hypothyroidism induction (30 days). FST in the first (B), second (C) and third (D) month of diphenyl diselenide supplementation after the hypothyroidism induction. Immobility time is expressed in seconds and represents the mean ± S.E.M. of 9–12 rats/group. *$p < 0.05$ from control group.

Se, MTZ and/or Se × MTZ interaction was found in these parameters ($p > 0.05$).

Fig. 2 shows the immobility time of groups during the FST. The data of Graph A reveal that the administration of MTZ in drinking water for 30 days caused a significant increase on the immobility time of animals when compared to control group (unpaired *T* Test, $p < 0.0001$); indicating that the experimental protocol of hypothyroidism was able to trigger a depressive-like behavior in FST.

Graphs B, C and D illustrate the effects of diphenyl diselenide on depression-like behavior induced by MTZ exposure after 1, 2 and 3 months of supplementation, respectively. Two-way ANOVA followed by Post Hoc comparisons indicated that MTZ exposed rats had a significant increase on the immobility time when compared to the control group in all periods of assessment ($p < 0.05$). In all periods analyzed, this effect of MTZ was completely blunted by dietary diphenyl diselenide ($p < 0.05$; Graphs B, C and D). A significant main effect of Se on immobility time was verified in the 1st [$F(1,44) = 13.68$, $p < 0.0006$], 2nd [$F(1,38) = 4.21$, $p < 0.04$] and 3rd [$F(1,44) = 5.54$, $p < 0.023$] month of treatment. Similarly, statistical analysis revealed a main effect of MTZ in all time periods (1st = [$F(1,44) = 14.91$, $p < 0.0004$]; 2nd = [$F(1,38) = 5.29$, $p < 0.027$] and 3rd = [$F(1,44) = 7.84$, $p < 0.007$]) on immobility parameter. Se × MTZ interaction was not significant on immobility time analysis ($p > 0.05$).

**Table 3**
Effects of MTZ and diphenyl diselenide supplementation on TBARS, ROS and NP-SH levels in the cerebral cortex, hippocampus and striatum.

| | | Control | MTZ | Se | MTZ + Se |
|---|---|---|---|---|---|
| Cerebral cortex | TBARS | 0.29 ± 0.04 | 0.24 ± 0.02 | 0.24 ± 0.03 | 0.19 ± 0.01 |
| | ROS | 7.27 ± 1.08 | 10.0 ± 3.18 | 8.98 ± 1.38 | 7.61 ± 1.52 |
| | NP-SH | 16.4 ± 1.32 | 13.1 ± 0.94 | 15.3 ± 0.87 | 16.1 ± 1.62 |
| Hippocampus | TBARS | 0.08 ± 0.02 | 0.26 ± 0.05* | 0.11 ± 0.01 | 0.17 ± 0.06 |
| | ROS | 5.52 ± 1.19 | 10.6 ± 0.93* | 6.59 ± 0.69 | 5.75 ± 1.01 |
| | NP-SH | 16.0 ± 1.02 | 12.6 ± 0.63 | 12.2 ± 0.49 | 15.44 ± 1.34 |
| Striatum | TBARS | 0.29 ± 0.03 | 0.27 ± 0.01 | 0.36 ± 0.05 | 0.11 ± 0.03 |
| | ROS | 26.4 ± 6.32 | 24.6 ± 6.51 | 21.6 ± 3.67 | 21.2 ± 2.4 |
| | NP-SH | 16.3 ± 1.02 | 11.9 ± 2.48 | 14.1 ± 1.86 | 16.0 ± 1.26 |

Values are expressed as means ± S.E.M. of 5–6 rats/group. TBARS are expressed as nmol malondialdehyde/mg of protein; ROS as μmol DCF/mg of protein and NP-SH as nmol SH/mg of protein.
* Denoted $p < 0.05$ from control group (two-way ANOVA followed Duncan's Test).

### 3.3. Lipid peroxidation, ROS and NP-SH determination

Two-way ANOVA followed by Post Hoc comparisons showed that MTZ exposure increased TBARS and ROS levels in the hippocampus when compared to the control group (Table 3). These effects of MTZ were restored to the control levels by diphenyl diselenide supplementation ($p < 0.05$). On the contrary, no statistical difference on lipid peroxidation and ROS levels in the cerebral cortex and striatum was verified among the groups. The NP-SH content was not modified by MTZ exposure and/or diphenyl diselenide supplementation in any of the cerebral structures analyzed (Table 3). No significant main effect of Se or MTZ was verified in these parameters ($p > 0.05$). However, there was a significant interaction between Se and MTZ in hippocampal ROS levels [$F(1,20) = 3.66$, $p < 0.007$].

### 3.4. MAO activities

Fig. 3 illustrates the MAO activities in total brain. Two-way ANOVA followed by Post Hoc comparisons revealed that MAO-total and MAO-A activities were not changed by MTZ exposure and/or dietary diphenyl diselenide when compared to the control group. Differently, MAO-B activity was significantly decreased in the MTZ group when compared to the control group ($p < 0.05$). Dietary diphenyl diselenide was effective in restoring MAO-B activity to the control levels ($p < 0.05$). Indeed, diphenyl diselenide did not modify per se the MAO activities. A

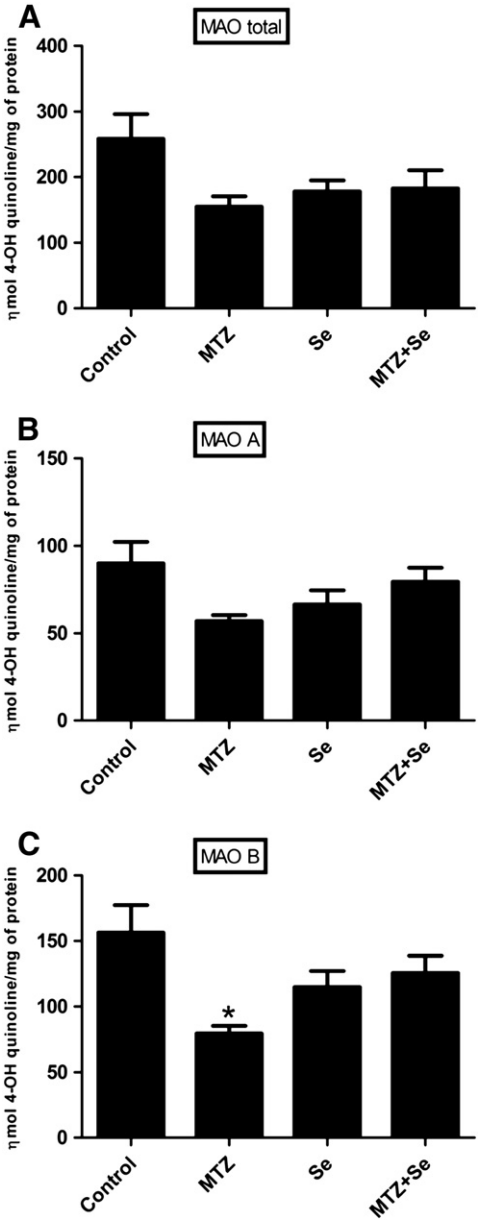



**Fig. 3.** Effects of MTZ and diphenyl diselenide supplementation on MAO activity. (A) MAO total activity, (B) MAO A activity and (C) MAO B activity. MAO activities were expressed as ηmol of 4-hydroxyquinoline formed/mg of protein and represent the mean ± S.E.M. of 5–6 rats/group.*$p < 0.05$ from control group.

significant Se × MTZ interaction was verified in both MAO A [$F(1,18) = 4.84$, $p < 0.04$] and MAO B [$F(1,17) = 7.89$, $p < 0.01$] activities.

## 4. Discussion

In the current study we have shown that the exposure of female rats to MTZ induced a depressive-like behavior that was substantially mitigated by diphenyl diselenide intake. The antidepressive-like effect associated to dietary diphenyl diselenide was obtained independently of thyroid hormone levels, which remained lower even in the hypothyroid group that received diphenyl diselenide.

The depressive-like behavior evaluation was performed by the FST that is widely employed as a behavioral tool for screening of antidepressant drugs [44]. The test is based on the observation that rodents, after initial escape-oriented movements, develop an immobile posture when exposed to an inescapable stressful event. In this way, when some antidepressant drug is administered prior to the test, the experimental subject will develop an escape-directed behavior for longer periods of time when compared to the control group. This task is quite sensitive to all major classes of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, monoamine oxidase inhibitors, and atypicals [51]. We also conducted an evaluation of locomotor and exploratory activities by open-field test in order to exclude mismatch factors to the forced swimming test. In this parameter, we observed that the reduction in the immobility time in the FST elicited by dietary diphenyl diselenide was not accompanied by alterations on locomotor activity, demonstrating that the effect exhibited by dietary diphenyl diselenide cannot be attributable to a psychostimulant action. Similarly, hypothyroidism condition did not change the locomotor and exploratory activities that could be harmful to animal performance in FST.

It is important to mention here that the chronic intake of diphenyl diselenide supplied diet usually is not associated with common signs of toxicity as body weight loss, hepatotoxicity and/or nephrotoxicity. Accordingly, previous works of our research group have shown that oral supplementation with this selenium compound (1–10 ppm) during similar periods may be considered relatively secure in terms of toxicity to rats and other species, including rabbits and fish [40–42,52–54]. However, under our experimental conditions, the animals treated with diphenyl diselenide alone exhibited T4 levels lower than the control. In view of the previous findings, we believe that this effect is due to the biologic variability found on thyroid status among the groups.

Hypothyroidism is an endocrine dysfunction linked to an increased susceptibility to depression and reduction in human health-related quality of life [6–9]. Epidemiological studies have demonstrated that severe and/or mild hypothyroidism induces depression symptoms [8,9]. Although, there is evidence on a possible interaction between monoaminergic systems and hypothalamic–pituitary–thyroid axis [13,55], the neuropharmacological basis and the functional pathways for the modulatory effects of thyroid hormones on mood are yet to be understood.

According to findings based on humans, experimental hypothyroidism causes depressive-like behavior. Kulikov et al. [37] have demonstrated that severe hypothyroidism induced by thyroidectomy or mild hypothyroidism caused by iodine-free diet is able to increase the immobility time of animals in the forced swimming paradigm. In analogy, our data confirm these results and also show that hypothyroidism triggered by methimazole exposure may be considered a consistent model to study this neurological disorder. Furthermore, we showed previously that hypothyroid female rats develop spatial learning and memory deficits [40].

The pharmacological properties of diphenyl diselenide are commonly linked to its antioxidant potential [25–29]. Nevertheless, there is evidence that antidepressant-like effect mediated by compound involves the participation of serotoninergic (5-HT1a, 5-HT2a/2c and 5-HT3 receptors), noradrenergic (α1 and α2 receptors) and dopaminergic (D1, D2 and D3 receptors) systems [21–23]. In terms of mechanisms it also highlights the role of some types of $K^+$ channels and PPARλ receptors (peroxisome proliferator-activated receptor) and the cerebral MAO activity modulation [22,56]. Indeed, the antidepressant-like effect of diphenyl diselenide verified by Acker et al. [24] on malathion-exposed rats was related with cerebral cortex $Na^+$–$K^+$–ATPase activity restoration. In our experimental protocol, the antidepressant-like action of compound observed on hypothyroid rats was followed by a reduction in hippocampal TBARS and ROS levels. Although we have not determined other oxidant/antioxidant parameters, it is possible assume here that the antioxidant activity of diphenyl diselenide had contributed to diminish the depression symptoms under hypothyroidism condition. Consistent with this finding, some studies have demonstrated the efficacy of antioxidant therapies in reducing stress oxidative parameters in experimental models of hypothyroidism [30–33].

In the current study we also investigate the MAO activity as a marker of oxidative stress and monoaminergic neurotransmission. MAO is a mitochondrial bound enzyme, which catalyzes the oxidative deamination of dietary amines, monoamine neurotransmitters and hormones. Two different types of MAO, named A and B, have been characterized on the basis of substrate and inhibitor sensitivity. MAO A displays a higher affinity for serotonin and norepinephrine, while MAO B prefers beta-phenylethylamine [57]. It is has been pointed that MAO B deficient mice present increased mobility in the FST without significant changes on locomotor patterns in the open-field and elevated plus maze tests [58]. Here, we found a significant inhibition of MAO B activity in hypothyroid rats. However, this activity reduced was accompanied by an increase on immobility time in FST test. Thus, it is possible to suppose that the inhibition of MAO B under hypothyroidism may reflect a compensatory response to oxidative insults and/or depressive behavior. In fact, the inhibition of MAO B activity could reduce oxidative stress and redox unbalance by diminishing the hydrogen peroxide formation as well as modulating mood by increasing the levels of beta-phenylethylamine, which acts as an endogenous amphetamine [58–60].

Taken together, our results show the efficacy of dietary diphenyl diselenide in declining the depressive-like behavior associated to methimazole-induced hypothyroidism in female rats. However, additional studies are necessary to better understand the mechanisms involved in the effects of both diphenyl diselenide and thyroid hormones on depression symptom development.

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