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Research report

Protective effect of *Melissa officinalis* aqueous extract against Mn-induced oxidative stress in chronically exposed mice

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ABSTRACT

Manganese (Mn) is an essential element for biological systems; however occupational exposure to high levels of this metal may lead to neurodegenerative disorders, resembling Parkinson's disease (PD). While its mechanisms of neurotoxicity have yet to be fully understood, oxidative stress plays a critical role. Thus, the main goal of this study was to investigate the efficacy of aqueous extract of *Melissa officinalis* in attenuating Mn-induced brain oxidative stress in mice. Sixteen male mice were randomly divided into two groups and treated for 3 months: the first group consumed tap water (control group) and the second group was treated with Mn (50 mg/kg/day for habituation during the first 15 days followed by 100 mg/kg/day for additional 75 days) in the drinking water. After 3 months both groups were sub divided ($n = 4$ per group) and treated for additional 3 months with Mn and/or *M. officinalis* in the drinking water. The first group (control) was treated with water and served as control; the second group (*M. officinalis*) was treated with *M. officinalis* (100 mg/kg/day); the third group was treated with Mn (100 mg/kg/day); the fourth group (Mn + *M. officinalis*) was treated with both Mn and *M. officinalis* (100 mg/kg/day each). Mn-treated mice showed a significant increase in thiobarbituric acid reactive species (TBARS) levels (a marker of oxidative stress) in both the hippocampus and striatum. These changes were accompanied by a decrease in total thiol content in the hippocampus and a significant increase in antioxidant enzyme activity (superoxide dismutase and catalase) in the hippocampus, striatum, cortex and cerebellum. Co-treatment with *M. officinalis* aqueous extract in Mn-treated mice significantly inhibited the antioxidant enzyme activities and attenuated the oxidative damage (TBARS and decreased total thiol levels). These results establish that *M. officinalis* aqueous extract possesses potent antioxidative properties, validating its efficacy in attenuating Mn-induced oxidative stress in the mouse brain.

1. Introduction

Manganese (Mn) is an abundant and essential metal acquired naturally through dietary intake [5]. Usually humans are exposed to low levels of Mn in air, food and water, which are kept at optimal concentration by both gastrointestinal absorption and efficient biliary excretion [7]. However, overexposure to Mn can also occur in occupational environments, and cases of Mn neurotoxicity (manganism) have been reported particularly in miners, smelters and workers in the alloy industry where exposures occur predominantly via the inhalation of Mn fumes or Mn-containing dusts [6]. The gasoline additive, methylcyclopentadyenylmanganese tricarbonyl (MMT), is another source of airborne Mn [32].

This essential trace element is required for normal growth, development, cellular homeostasis and many ubiquitous enzymatic reactions involved in neurotransmitter synthesis and metabolism [5,44]. However, at high doses it has been considered a neurotoxic metal [28,41]. Chronic exposure to Mn leads to excessive Mn accumulation in the nervous system [9,22], predominantly in the basal ganglia, namely in the globus pallidus, striatum and substantia nigra pars reticulata [16,48]. As a result, Mn induces a decrease in dopamine (DA) levels and cell death, a syndrome commonly referred to as manganism [10], resembling idiopathic Parkinson's disease (PD).

The cellular and molecular mechanisms of Mn-induced neurotoxicity have yet to be fully understood. Mn exerts its cellular toxicity via several mechanisms, including direct or an indirect

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formation of reactive oxygen species (ROS) [8,36], oxidation of biological molecules [4] and the disruption of Ca^{2+} and iron (Fe) homeostasis [25,66]. An imbalance between ROS generation and antioxidant defense mechanisms with subsequent oxidative stress [26,27] may initiate apoptosis and/or necrosis [40]. Thus, oxidative stress is a putative mechanism by which Mn induces neuronal damage [20], and its mediation of neuronal damage is further supported by the protective effect of *N*-acetylcysteine (NAC), glutathione (GSH) and vitamin C in mitochondrial preparations against Mn-induced ROS production [29,65]. Accordingly, it is reasonable to postulate that natural biomolecules possessing antioxidant properties may be effective in attenuating Mn-induced oxidative stress.

Melissa officinalis belongs to the Laminaceae family, being a perennial herb. Preparations derived from the aerial part of *M. officinalis* are often used in folk medicine for the treatment of fevers and colds, indigestion associated with nervous tension, hyperthyroidism, depression, mild insomnia, epilepsy, headaches, toothaches, flatulence, colic, nausea, nervousness, anaemia, vertigo, syncope, malaise, asthma, bronchitis, amenorrhea, cardiac failure, arrhythmias, depression, psychosis, hysteria, ulcers, wounds, among others [14,52]. In addition, recent data from literature have supported a protective role for *M. officinalis* intake against Alzheimer disease [53].

M. officinalis extracts possess antioxidant [14,42], sedative [33], anti-inflammatory, hepatoprotective, digestive [54,56], antiviral [14,3], antilipidaemic [12] and anxiolytic [53] properties. In addition, *M. officinalis* extracts show efficacy in ameliorating some symptoms of Alzheimer's disease (AD) [2].

Phytochemical studies carried out in *M. officinalis* have demonstrated the presence of numerous constituents, including polyphenolic compounds, essential oils, monotherpenoid aldehides, sesquiterpenes, flavonoids and tannins [14,52,33,19]. All of these may be responsible for the therapeutic efficacy of *M. officinalis* extracts and the prevention of the effects described above.

Considering the major role of oxidative stress in Mn-induced neurotoxicity and the presence of a number of compounds with antioxidant properties in *M. officinalis* plant extracts, we hypothesized that treatment with the aqueous extract of this plant would result in protective effect against Mn-induced neurotoxicity in a long-term mouse intoxication model. Additionally, we chose this herb over other potential botanical therapies or over bioactive compounds (e.g., catechins, flavonols, etc.) found in food due to two reasons: (a) recently we have shown a more pronounced antioxidant effect of *M. officinalis* against three different pro-oxidants, under in vitro conditions, compared to *Matricaria recutita* and *Cymbopogon citratus* [42]; (b) the antioxidant effect of *M. officinais*, in vivo, are very scarce in the literature. Considering both, we decide to test if the well reported in vitro antioxidant properties of *M. officinalis*, could also be found under in vivo conditions, using a Mn-chronically exposed mice model.

2. Materials and methods

2.1. Chemicals

Manganese chloride ($MnCl_2$), 2-thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), epinephrine and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma, St. Louis, MI, USA. All the other chemicals were commercial products of the highest purity grade available.

2.2. Animals

Young male albino mice (20–25 g) from our own breeding colony were used in this study. The animals were housed in plastic cages with water and food (Guabi-RS, Brasil) ad libitum, at 22–23 °C, humidity approximating 56%, and 12/12 (h) light/dark cycle. The animals were used according to guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.

2.3. Preparation of the M. officinalis aqueous extract

The plants were obtained from commercial sources (Madrugada Alimentos Ltda, Venâncio Aires, RS, Brazil). Aqueous extracts were obtained by maceration of 500 mg of dried plant material (aerial parts) placed in 100 mL hot water (100 °C; for 10 min). The extracted solution was filtered through filter paper and its concentration was adjusted with distilled water. In other words, the initial 100 mL of hot water diluted to 1.667 L, resulting in 30 mg of plant in 100 mL of water.

2.4. Animals' treatments

Sixteen animals were divided into two groups with 8 animals each. Group 1 was treated daily for three months with tap drinking water; group 2 was treated for the first 15 days with 50 mg/kg of $MnCl_2$ dissolved in the drinking water (15 mg of $MnCl_2$ in 100 mL of water) followed by 100 mg/kg of $MnCl_2$ (30 mg of $MnCl_2$ in 100 mL of water) for the next 75 days. Water consumption was monitored every 2 days in order to correct the Mn dosage, when necessary. Body weight gain was monitored every 2 weeks. During first 90 days the animals were weekly accompanied for the sings of Mn intoxication in the open field apparatus. We clearly noticed that Mn exposed mice presented a decrease in the locomotor and exploratory activity, compared to control mice (data not shown); because after four experimental sections the animals became adapted to apparatus and no significant differences were seen among groups. After three months, both groups (1 and 2) were randomly divided into four groups containing 4 animals each. Subsequent treatments were as follows:

(1) Control (only water).
(2) *M. officinalis* control (treated with *M. officinalis* aqueous extract 100 mg/kg/day in the drinking water for additional 90 days).
(3) Mn ($MnCl_2$ in drinking water for additional 90 days).
(4) Mn + *M. officinalis* ($MnCl_2$ + *M. officinalis* 100 mg/kg/day in drinking for additional 90 days).

The administered doses of Mn and *M. officinalis* aqueous extract were based on previous studies ([9,15], respectively). Additionally, Mn-chelating properties of the *M. officinalis* aqueous extract was investigate as previously described [55] after mixture both (Mn and *M. officinalis* extract) in different proportions and time of co-incubation. In fact, we found that *M. officinalis* aqueous extract does not interact with Mn under our experimental conditions (data not shown), suggesting that *M. officinalis* extract was not able to decrease the intestinal absorption of Mn via direct chelating effects.

2.5. Tissue preparation

At the end of the treatments, mice were euthanized, the brains were removed and the cortex, cerebellum, hippocampus and striatum were dissected out and homogenized (1:10) in 10 mM Tris-buffer (pH 7.4) followed by centrifugation (10 min, 2000 × *g*, 4 °C) to obtain a low speed supernatant (S1) for the biochemical analysis.

2.6. Thiobarbituric acid reactive substances production (TBARS)

Lipid peroxidation in the S1 of the cortex, cerebellum, hippocampus and striatum were analyzed by the thiobarbituric reactive substances (TBARS) method [39]. TBARS were determined spectrophotometrically at 532 nm after 1 h of incubation of 200 μL of S1 from the various brain areas with SDS 8.1%, acetic acid/HCl buffer and thiobarbituric acid 0.6% at 95 °C.

2.7. Total thiol determination

Although the specific molecular targets by which Mn-induces oxidative stress are not known, it has been reported that Mn can indirectly interact with low molecular weight thiols via Mn-induced ROS generation, oxidizing them to disulfides [18]. Consequently, total thiol content in cortex, cerebellum, hippocampus and striatum were determined in S1 fraction of the samples with the Ellman's reagent (DTNB), measured spectrophotometrically at 412 nm, following standard methodology [21].

2.8. Superoxide dismutase (SOD) activity

SOD was determined in S1 fraction of cortex, cerebellum, hippocampus and striatum following the method by Misra and Fridovich [38]. The adrenochrome production was measured spectrophotometrically at 480 nm. One unit of the enzyme was defined as the amount of enzyme required to inhibit the rate of adrenaline auto-oxidation by 50%.

2.9. Catalase (CAT) activity

CAT activity in S1 of cortex, cerebellum, hippocampus and striatum was analyzed following the method by Aebi [1], measuring the rate of disappearance of H_2O_2 spectrophotometrically at 240 nm. One unit of the enzyme was considered as the amount which decomposes 1 μmol H_2O_2/min at pH 7.

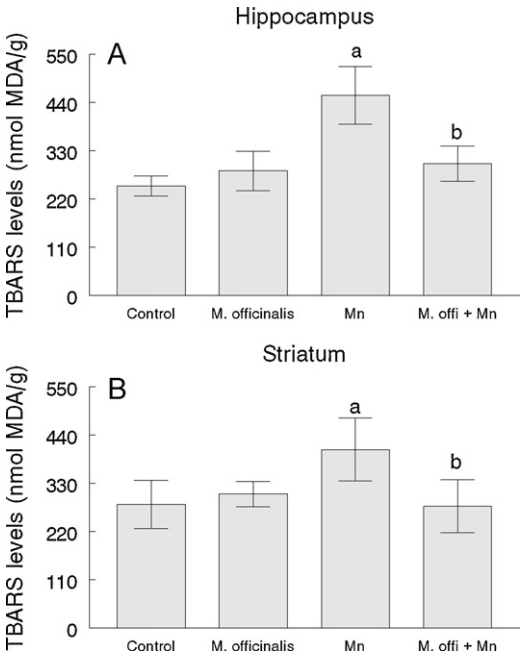

Fig. 1. Effects of Mn treatment (100 mg/kg/day) and/or co-treatment with *M. officinallis* (100 mg/kg/day) on TBARS levels in mouse hippocampus (A) and striatum (B). Data are expressed as nmols of malondialdehyde (MDA)/g of tissue. Each bar represents mean ± S.E.M. ($n = 4$). (a) Statistical difference from the control group; (b) Statistical difference from the Mn-treated group by two-way ANOVA, followed by Duncan *post hoc* test ($p < 0.05$) when appropriate.

2.10. Protein determination

Aliquots from the homogenates were separated for protein measurements that were assessed according to Bradford [13]. Results for each of the biochemical assays were corrected for protein content in the samples.

2.11. Statistical analysis

Biochemical data were analyzed by two-way ANOVA followed by Duncan's multiple range test when appropriate, and the values were expressed as mean ± S.E.M. Statistical software package for Windows version 8.0 was used and $p < 0.05$ was considered as statistically significant for all comparisons made.

3. Results

Mn treatment caused a significant increase in TBARS levels in the hippocampus and striatum ($p < 0.05$) (Fig. 1A and B, respectively), but not in the cortex and cerebellum (Table 1). Moreover, statistical analysis indicated a significant ($p < 0.05$) interaction between Mn and *M. officinalis*, either in hippocampus and striatum, but not in the cortex and cerebellum. Co-treatment with *M. officinalis* significantly ($p < 0.05$) attenuated the increase in Mn-induced TBARS levels in the hippocampus and striatum to levels indistinguishable from controls (Fig. 1A and B). Treatment with *M. officinalis* alone had no effect on brain TBARS levels (Fig. 1A and B) in any of the brain areas.

Table 1
TBARS levels in cortex and cerebellum in Mn-treated mice: protection with *M. officinalis* treatment.

	Cortex	Cerebellum
Control	277.89 ± 39.59	320.13 ± 42.27
M. officinalis	310.54 ± 45.40	369.05 ± 18.66
Mn	276.31 ± 21.11	353.76 ± 46.48
Mn + *M. officinalis*	295.60 ± 56.02	323.78 ± 51.74

Data are expressed as mean ± S.E.M. ($n = 4$). Values are expressed as nmol MDA/g. Data were analyzed by two-way ANOVA following by Duncan *post hoc* test when appropriate.

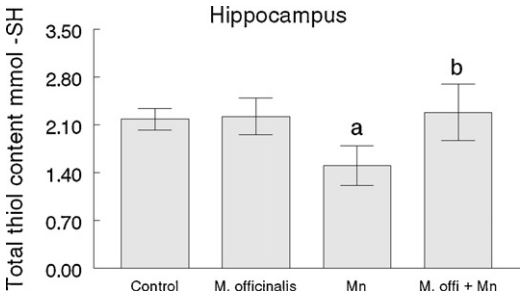

Fig. 2. Effects of the Mn exposure (100 mg/kg/day) and/or co-treatment with *M. officinallis* (100 mg/kg/day) on total thiol content in mouse hippocampus. Data are expressed as nmols of –SH/g of tissue. Each bar represents mean ± S.E.M. ($n = 4$). (a) Statistical difference from control group by two-way ANOVA, following by Duncan *post hoc* test ($p < 0.05$) when appropriate.

Table 2
Total thiol content in striatum, cortex, and cerebellum in Mn-treated mice: protection with *M. officinalis* treatment.

	Striatum	Cortex	Cerebellum
Control	2.76 ± 0.09	2.65 ± 0.12	2.43 ± 0.11
M. officinalis	2.67 ± 0.10	2.67 ± 0.06	2.57 ± 0.29
Mn	2.81 ± 0.11	2.41 ± 0.19	2.48 ± 0.15
Mn + *M. officinalis*	2.78 ± 0.14	2.39 ± 0.31	2.47 ± 0.23

Data are expressed as mean ± S.E.M. ($n = 4$). Values are expressed as nmol –SH/g. Data were analyzed by two-way ANOVA following by Duncan *post hoc* test when appropriate.

Consistent with increased ROS generation, Mn treatment caused a significant ($p < 0.05$) decrease in total thiol content, but this effect was inherent only to the hippocampus (Fig. 2; Table 2). The Mn-induced decrease in hippocampal thiol content was completely restored by *M. officinalis* treatment (Fig. 2) to levels indistinguishable from controls. However, statistical analysis did not indicated significant interaction between Mn and *M. officinalis* in hippocampus. Treatment with *M. officinalis* alone had no discernable effect on thiol content in any of the investigated brain areas (see Fig. 2 and Table 2).

SOD activity was significantly ($p < 0.05$) increased by Mn treatment in all the investigated structures (hippocampus, striatum, cortex and cerebellum) (see Fig. 3A, B and Table 3). Moreover, statistical analysis indicated a significant ($p < 0.05$) interaction between Mn and *M. officinalis* in cortex. Treatment with *M. officinalis* extract completely restored SOD activity to control levels in the hippocampus (Fig. 3A), but only partially in the striatum (Fig. 3B), cortex and cerebellum (Table 3), all three remaining statistically ($p < 0.05$) significantly different from the control group.

CAT activity was significantly ($p < 0.05$) increased by Mn treatment in all the studied brain areas (Fig. 4A, B and Table 4). Moreover, statistical analysis indicated a significant ($p < 0.05$) interaction between Mn and *M. officinalis* only in cortex. However, *M. officinalis* treatment did not attenuate CAT activity in the hippocampus (Fig. 4A) and only partially restored it in the striatum (Fig. 4B)

Table 3
SOD activity (UI/min) in cortex and cerebellum of Mn-treated mice: Protection with *M. officinalis* treatment.

	Cortex	Cerebellum
Control	5.10 ± 0.93	6.44 ± 1.23
M. officinalis	7.30 ± 2.20	4.50 ± 1.65
Mn	27.31 ± 2.43[a]	21.26 ± 2.93[a]
Mn + *M. officinalis*	12.33 ± 1.06[a,b]	11.20 ± 1.77[a,b]

Data are expressed as mean ± S.E.M. ($n = 3$–4).
[a] Different of control group.
[b] Different of Mn group by two-way ANOVA following by Duncan *post hoc* test when appropriate.

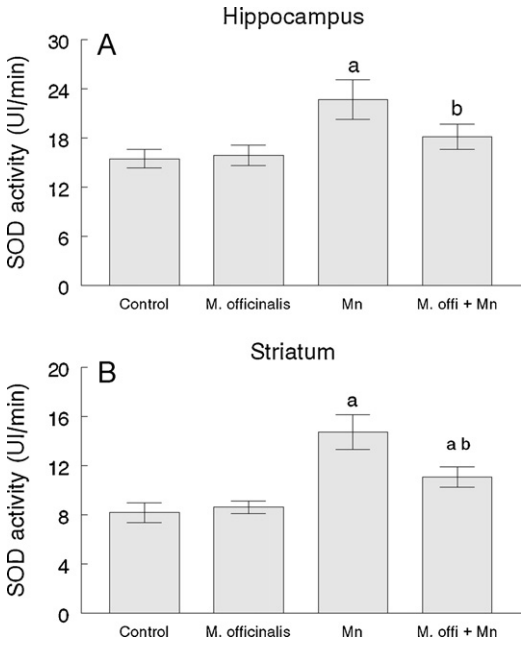

Fig. 3. Effects of the Mn exposure (100 mg/kg/day) and/or co-treatment with *M. officinallis* (100 mg/kg/day) on SOD activity in mouse hippocampus (A) and striatum (B). Data are expressed as UI/min. Each bar represents mean ± S.E.M. ($n = 4$). (a) Statistical difference from control group; (b) statistical difference from Mn-treated group by two-way ANOVA, following by Duncan *post hoc* test ($p < 0.05$) when appropriate.

and cortex (Table 4), remaining statistically ($p < 0.05$) significantly different from the control group. However, in the cerebellum, *M. officinalis* treatment completely restored CAT activity in Mn-treated mice (Table 4) to levels indistinguishable from control group.

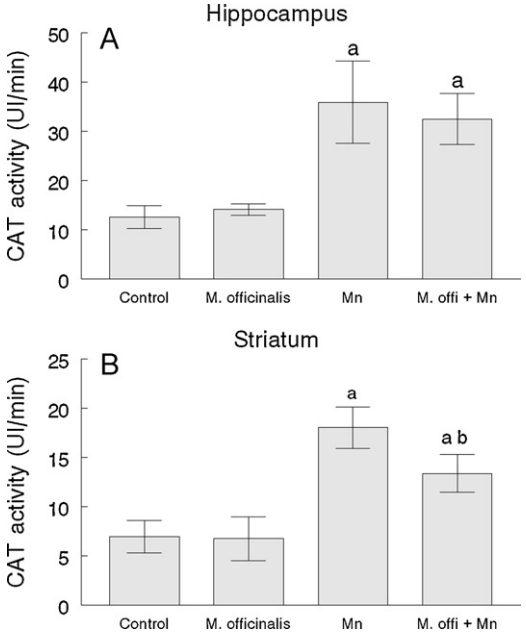

Fig. 4. Effects of the Mn exposure (100 mg/kg/day) and/or co-treatment with *M. officinallis* (100 mg/kg/day) on CAT activity in mouse hippocampus (A) and striatum (B). Data are expressed as UI/min. Each bar represents mean ± S.E.M. ($n = 4$). (a) Statistical difference from control group; (b) statistical difference from Mn-treated group by two-way ANOVA, following by Duncan *post hoc* test ($p < 0.05$) when appropriate.

Table 4
CAT activity (UI/min) in cortex and cerebellum of Mn-treated mice: protection with *M. officinalis* treatment.

	Cortex	Cerebellum
Control	6.60 ± 0.38	5.11 ± 0.99
M. officinalis	8.33 ± 0.77	6.03 ± 0.61
Mn	13.00 ± 1.04[a]	17.36 ± 6.14[a]
Mn + *M. officinalis*	10.42 ± 1.35[a,b]	7.92 ± 1.11[b]

Data are expressed as mean ± S.E.M. ($n = 3$–4).
[a] Different of control group.
[b] Different of Mn group by two-way ANOVA following by Duncan *post hoc* test when appropriate.

4. Discussion

Human exposure to Mn is of clinical interest because of the neurological symptoms that it can cause after exposure in occupational and/or environmental settings. In fact, a recent study suggests that high levels of Mn in drinking water (>300 μg/L) are associated with reduced intellectual function [62], although additional exposure (food and air containing Mn) could have pushed the total daily dose above that value. The syndrome caused by Mn toxicity shares similar characteristics to PD, and accordingly it has been postulated that Mn exposure is a risk factor for this disease [47]. In this context, recent studies have indicated that oxidative stress plays a key role in Mn-induced neurotoxicity [9,8,35,37,50]. In fact, oxidative stress has been implicated in the pathophysiology of numerous neurodegenerative disorders [11,61]. In the present study, mice treated with Mn in the drinking water (mimicking an environmental chronic exposure to high Mn levels) showed increased lipid peroxidation (as measured by a significant increase in TBARS levels) both in the hippocampus and striatum (Fig. 1A and B), a decrease in total thiol content only in the hippocampus (Fig. 2) and an increase in the antioxidant enzymes (SOD and CAT) in the hippocampus, striatum, cortex and cerebellum (Figs. 3 and 4, Tables 3 and 4). Remarkably, the majority of the Mn-induced effects on the oxidative stress parameters were partially or fully reversed by co-treatment with the *M. officinalis* aqueous extract. However, Mn content in the brain regions was not determined; therefore it is unknown whether the reported effects are due to *M. officinalis* preventing Mn accumulation or directly effecting enzyme activity in affected brain regions.

The effects of Mn were brain area-dependent. For example, the effect on TBARS was restricted to the hippocampus (Fig. 1A) and striatum (Fig. 1B) with a maximal increase of 1.4–1.8-fold. For the total thiol content, the Mn-induced decrease was relatively small (about 30%) and it was restricted to the hippocampus (Fig. 2). In contrast, CAT and SOD activities were increased 1.5–5.0 times after Mn treatment, dependent on the brain area. The Mn-induced increase in SOD and CAT activity was more pronounced in the cortex and cerebellum (Tables 2 and 3) compared to hippocampus and striatum (Figs. 3 and 4). Collectively, these results indicate a heterogeneous effect of Mn, which is dependent upon both the molecular endpoint and the analyzed brain structure. In fact, Mn did not lead to a ubiquitous effect in all brain regions, but rather, selective oxidative stress in those areas known to be predominately sensitive to this metal [7,6,9,43].

In sharp contrast, the effect of *M. officinalis* extract was generally homogenous in all brain areas and for all the analyzed molecular endpoints. Indeed, we demonstrate for the first time, an in vivo antioxidant efficacy for the *M. officinalis* aqueous extract against Mn-induced oxidative stress in the mouse brain. Co-treatment with the plant extract in the drinking water at 100 mg/kg/day completely attenuated the Mn-induced lipid peroxidation (TBARS) in the hippocampus (Fig. 1A) and striatum (Fig. 1B) as well as the increase in total thiol content in the hippocampus (Fig. 2). These

findings are likely due to the antioxidant activity of *M. officinalis* aqueous extract, which has only been reported in vitro prior to the present study [14,42]. Thus, taking into account the literature data, we posit that the plant's constituents (mainly flavonoids) may be responsible for the protective effects of *M. officinalis* aqueous extract [49,59,63].

In agreement with our studies, earlier reports in human post-mortem brains, non-human primates and rodents corroborate Mn-induced neuronal damage is most prominent in the hippocampus and striatum [6,43,17]. We suggest that the striatum and hippocampus are more sensitive to Mn due to a higher content of divalent metals transporters type-1 (DMT-1) in these areas compared to others [7,9]. Further evidence of Mn accumulation in the hippocampus is provided by previous data, showing that 3 days post injection, Mn was localized to the dentate gyrus and CA3 of the hippocampus [6,58]. Additionally, the striatum contains higher DA content than other brain areas, which in the presence of Mn(II) may lead to increased ROS generation [23,45] contributing to oxidative stress related changes. We hypothesize that the Mn-induced oxidative changes (increase in TBARS and antioxidant enzymes SOD and CAT and the decrease in total thiol content) in the hippocampus and striatum reported here must be, at least in part, due to the preferential accumulation of Mn in these brain areas, as compared to the cerebellum and cortex. This conclusion however, does not account for the fact that activities of SOD were higher induced in cortex and cerebellum compared to hippocampus and striatum after Mn exposure. Taking this into account, we speculate that some kind of deficiency of SOD activation mechanisms following Mn exposure in cerebellum and cortex may play an important role in selective sensitivity of these two regions to Mn-induced oxidative stress.

Additionally to DMT-1, we must consider the differential expression of ferroportin-1 under Mn exposure. In fact, ferroportin-1 is an iron exporter that shows altered surface localization following Mn exposure, making it a potential candidate as a putative Mn exporter [60]. In view of that, it was recently shown that mice exposed to Mn showed an increase in ferroportin-1 levels in both cerebellum and cortex [64], contributing to reduced cytotoxicity associated to the exposure to this metal. In fact, one could speculate that under our experimental conditions the increase in the levels of ferroportin-1 levels in both cerebellum and cortex associated with lower content of DMT-1 could be responsible for the lower sensitivity of both structures reported here; whereas lower levels of ferroportin-1 and higher content of DMT-1 in striatum and hippocampus could contribute to the higher sensitivity observed in both structures. However, whether such differences (relatively low ferroportin-1 and relatively high DMT-1 expression levels) account for the propensity of hippocampal and striatal areas to accumulate large amounts of Mn remains to be established.

Reduced cysteinyl residues from proteins may be a molecular target for Mn toxicity, which might cause the loss of enzyme catalytic activities [46]. Thus, considering that GSH is the major naturally occurring nonenzymatic antioxidant in the brain [24], the potential indirect oxidation of –SH groups by Mn-induced ROS may cause depletion of –SH groups, contributing to a decrease in the non-enzymatic antioxidant defenses in hippocampus of treated mice reported in the present investigation (Fig. 2). It is therefore presumed that the decrease in total thiol levels reported here were due to GSH, that could be oxidized due either to the excess of free radical formation or by regenerating the nitrosyl groups [57,51]. In fact, GSH was shown to provide cell protection, generating GSSG and protein S-glutathionylated adducts (–SSG) [34]. However it does not address the specificity of the Mn-induced effect. Thus, we posit that Mn-induced ROS generation may be responsible for the decrease in the hippocampal thiols (Fig. 2). However, unexpectedly the total striatal thiol content was not changed after Mn treatment (Table 2), suggesting that Mn-induced thiol oxidation is a complex process that could not be easily explained by solely taking into account the Mn-induced ROS generation.

As mentioned above, Mn modulated the activity of the antioxidant enzymes by increasing SOD and CAT in all the studied brain areas (Figs. 3 and 4, Tables 3 and 4). Notably, these enzymes represent the first barrier against reactive species and are essential to cell survival [26,27]. We speculate that the observed increase in the antioxidant enzymes (SOD and CAT) may be due to an adaptive response, which mitigates the Mn-induced oxidative damage [30]. Thus, after prolonged exposure, Mn may lead to an adaptive mechanism, as evidenced by increased activity of these enzymes [31]. *M. officinalis* aqueous extract partially restored the activities of SOD and CAT in all studied brain areas, likely due to its antioxidant effects.

5. Conclusions

In summary, our results clearly demonstrated that antioxidant properties of *M. officnalis* may be potentially neuroprotective against Mn-induced neurotoxicity, especially in the hippocampus and striatum. However, further studies are required to identify the active constituents involved in the antioxidant and neuroprotective activity of this plant. In addition, studies on uptake of the active ingredients of *M. officinalis* must be performed to better understand its potential utility as a broad spectrum therapeutic antioxidant in manganism as well as other neurodegenerative disorders.

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