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Research Article

Effects of Pentoxifylline in a Rat Model of Manganism: Evaluation of the Possible Toxicity

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Objective. Manganese (Mn) has been reported, through dietary and occupational overexposure, to induce neurotoxicity named manganism. Pentoxifylline (PTX) administration attracts much attention considering the beneficial properties of PTX, as an anti-inflammatory and smooth muscle relaxation agent. This *in vivo* study aims to evaluate the effect of PTX on manganism in rat model. *Materials and Methods*. Thirty adult male Sprague Dawley rats received $MnCl_2$ (100 mg/kg, i.p. on days 1, 3, and 7) during a week alone or in combination with PTX (300 mg/kg, i.p. every day for 8 consecutive days on manganism rat model). Several locomotor activity indices, as well as biomarkers of oxidative stress, were monitored in the brain tissue of Mn-exposed animals. *Results*. It was found that PTX supplementation (300 mg/kg, i.p.) deteriorated the Mn-induced locomotor deficit. This drug also increased the Mn brain accumulation as well as reactive oxygen species (ROS) and lipid peroxidation products in the manganism rat model. Moreover, the levels of total antioxidant capacity (TAC) and glutathione (GSH) were shown to be reduced significantly compared to the control group. *Conclusion*. The results of this study revealed that PTX at a high dose (300 mg/kg) might increase manganism complications. PTX lowers the blood viscosity, improves the tissue perfusion, and increases the Mn levels in the brain.

1. Introduction

Manganese (Mn) is an essential metal ion present in the environment, including in water, soil, and food. Mn is critical for normal growth, development, bone mineralization, cellular energy, and homeostasis. Also, Mn plays a significant role in the optimal function of multiple enzymes named glutamine synthetase, arginase, and pyruvate decarboxylase [1]. Although Mn deficiency rarely happens, it can result in birth defects and seizures [2]. It was reported that exposure to high levels of Mn might result in brain accumulation, neurotoxicity, and neurodegeneration, named manganism [3]. Manganism disorder is characterized by behavioral changes, including irritability, aggressiveness, slow and clumsy movements, tremors, difficulty walking, facial muscle spasms, and hallucinations [4]. Importantly, due to the new lifestyle and industrial society in recent decades, metal, especially Mn, exposure has drawn public attention [5]. Mn toxicity happened due to repeated nutritional, environmental, and occupational exposures, especially in miners, smelters, and welders [6]. Also, in the industrialized world, new Mn-containing chemicals will inevitably be synthesized, and novel applications will be established [7]. Unlike many of the metals, Mn can easily be found in an everyday meal including in nuts, grains, chocolate, mussels, soybeans, seeds, and spices which are accumulated in the body [8]. What makes the problem worse is that patients exposed to Mn may be asymptomatic for months or years. Several recent reports have focused on potential risk factors of Mn on different diseases. Various studies have confirmed that the patients who have Parkinson's disease (PD), Parkinsonism, or Alzheimer's disease

(AD) have a history of Mn exposure [9]. Although the pathophysiology of manganism is complex, it has been found that Mn can easily cross the blood-brain barrier by facilitating diffusion and/or active transport, leading to accumulation of Mn in brain regions, especially in the basal ganglia; it targets adrenergic neuron and cholinergic system which induces neurotoxicity [10].

Pentoxifylline (PTX), with the formula of 1-(5-oxohexyl)-3, 7-dimethylxanthine, is the most established drug therapy agent that is most commonly used for the treatment of intermittent claudication, peripheral vascular disease, cerebrovascular disease, and several other conditions involving a defective regional microcirculation [11, 12]. It has been proposed that PTX affects the function of white blood cells and hemorheological parameters. PTX also demonstrated a significant inhibitory activity effect against phosphodiesterase, tumor necrosis factor-alpha (TNF-α), and the transcription factor NF-κB, making it an ideal therapeutic candidate for inflammatory diseases including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and psoriasis alongside blood thinners potential in humans [13–15].

With growing interest in consuming PTX among people, however, it remains unclear whether PTX medication has adverse effects on manganism and Mn-exposed workers, especially in industrial areas. The objective of this study was to investigate the effects of PTX on manganism in rat model.

2. Materials and Methods

2.1. Chemicals. Thiobarbituric acid (TBA), 2,4-dinitro fluorobenzene (DNFB), manganese chloride, trichloroacetic acids (TCA), taurine, and hydroxymethyl aminomethane hydrochloride (Tris–HCl) were purchased from Merck (Darmstadt, Germany). All salts for making buffer solutions were of analytical grade and were prepared from Merck (Darmstadt, Germany). Reduced glutathione (GSH) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals. All the procedures were in agreement with the guidelines of the Committee for Research Ethics of the Shiraz University of Medical Sciences (no. 98-01-36-19479). Thirty adult male Sprague Dawley rats aged 10–12 weeks and weighing between 200 ± 20 g were purchased from the animal laboratory center of Shiraz University of Medical Sciences. The rats were maintained under standard housing laboratory conditions, relative humidity of $55 \pm 5\%$, temperature of $22 \pm 2^{\circ}$C, and 12-h light/dark cycles. Rats were allowed free access to standard food and water *ad libitum*. The food ingredients are shown in Table 1.

2.3. Animal Model of Manganism. After one week of adaptation to the new environment, the rats were randomly divided into three groups ($n = 10$ in each group):

Group 1, or control, received vehicle-treated i.p. every day for 1 week

Group 2 received three subcutaneous doses of 100 mg/kg of $MnCl_2$ (days 1, 3, and 7 during a week)

Group 3 received three subcutaneous doses of 100 mg/kg of $MnCl_2$ (days 1, 3, and 7 during a week)- + pentoxifylline (300 mg/kg, i.p for 8 consecutive days) [16, 17]

24 hours after the final dose of Mn, locomotor activity tests were assessed [17, 18]. On day 9, brain tissue markers of oxidative stress were monitored, and mitochondrial functionality indices were evaluated. At the end of the study, the rats were euthanized with the rapid and humane method, using a 70% volume displacement rate of CO_2 increased to 100% in the induction chamber. After the rats were sacrificed, their brains were immediately dissected from the skull and weighed; the homogenates (10% w/v) were prepared using a glass homogenizer with ice-cold 1.15% KCl. Brain tissue can also be frozen in liquid nitrogen and stored at -80°C until assayed.

2.4. Rotarod Test. According to the previously reported procedure [18, 19], each rat underwent five sessions of rotarod performance on a rotarod apparatus. The speed of the rotarod was 5 and 15 rpm (cutoff point: 300 sec). The time, up to which the animal stayed on the rotating rod, was automatically recorded.

2.5. Gait Test. Gait test was performed based on a reported procedure [19, 20]. Briefly, the rat hind paws were wetted with ink. Afterward, using a runaway procedure, the rats were allowed to walk down on a paper strip (60 cm long, 10 cm wide) from the brightly lit corridor toward a dark side. The distance between the points of the left and right hind paws was measured.

2.6. Plasma and Brain Mn Level. Rat plasma and brain Mn levels were measured based on inductively coupled plasma mass spectrometry (ICP-MS). Briefly, plasma (500 μL) and tissue samples (500 μL of 10% w: v tissue homogenate) were treated with 100 μL of nitric acid (HNO_3; 2.5% w: v) and centrifuged (10,000 g, 10 min). The supernatants were transferred to new tubes and used for Mn determination. Mn concentration is indicated in μg/g protein for the brain tissue and μg/mL for plasma samples.

2.7. Markers of Oxidative Stress in the Brain Tissue

2.7.1. Total Antioxidant Capacity (TAC). The cerebral cortex of the brain tissue (200 mg) was homogenized in 2 mL of an ice-cooled buffer (5 mm potassium phosphate, 0.9% sodium chloride, and 0.1% glucose, pH = 7.4; 4°C) and centrifuged at 10.000 g for 15 min at 4°C; then, the supernatant was removed for the assay. The total antioxidant capacity (TAC) was evaluated by colorimetric method using a commercial kit (Cayman, USA).

Table 1: Ingredient composition of the diets fed to the rats (g/100 g).

Ingredients	Diet
Protein mix	23
Mineral mix	12
Fiber	5
Vitamin mix	4–4.5
Fat	10
Fatty acids	5
Corn starch	32
Sugar mix	6
Wheat bran	3
Soybean meal	3

2.7.2. Lipid Peroxidation. As previously described, the thiobarbituric acid reactive substances (TBARS) test was used as a method to assess lipid peroxidation in the brain tissue [21]. Brain tissue samples of the forebrain (cerebral cortex) were dissected; 500 μL of tissue homogenate (10% w/v in Tris-HCl buffer 40 mm, pH = 7.4), 3 mL of metaphosphoric acid (1% w/v, pH = 2), and 1 mL of thiobarbituric acid (0.375%, w/v) were poured into the test tube, mixed well, and heated in a water bath (100°C; 45 min). Then, the mixture was cooled, and 2 mL of *n*-butanol was added. Samples were vortexed for 5 min and centrifuged (10,000 g for 5 min). Finally, the absorbance of the color developed in *n*-butanol (upper phase) was measured at $\lambda = 532$ nm, using a BioTek plate reader (BioTek®, Highland Park, USA) [17].

2.7.3. Reactive Oxygen Species Formation. Reactive oxygen species (ROS) formation in the brain tissue was estimated based on a procedure using 2′,7′-dichlorofluorescein diacetate (DCF-DA) as a fluorescent probe [22, 23]. Brain tissue samples of the forebrain (cerebral cortex) were dissected, weighed, and then sonicated in 1 mL of ice-cold 40 mm Tris-HCl buffer (pH 7.4) and 10 μL of DCF-DA (final concentration 10 μm). The mixture was incubated at 37°C for 15 min in dark. Finally, the fluorescence intensity (FI) of the samples was assessed with λ excitation = 485 nm and λ emission = 525 nm, using a POLARstar Omega® (BMG Labtech, Germany) microplate reader.

2.7.4. Glutathione Content. Brain tissue-reduced glutathione (GSH) was measured spectrophotometrically using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as the indicator. More specifically, brain tissue samples of the forebrain (cerebral cortex) were dissected, and 200 mg of brain tissue was homogenized in 8 mL of ice-cooled EDTA solution (20 mm). Then, 5 mL of the homogenized sample was mixed with 4 mL distilled water and 1 mL trichloroacetic acid (50% w/v). The mixture was gently shaken and centrifuged (10,000×g, 10 min, 4°C). Then, 2 mL of the supernatant was mixed with 4 mL Tris buffer (pH = 8.9) and 100 μL Ellman's reagent (DTNB, 10 mm in methanol). The intensity of the developed yellow color was measured at $\lambda = 412$ nm, using POLARstar Omega® (BMG Labtech, Germany) microplate reader [24].

2.8. Statistical Analysis. The SPSS statistical software (v. 18) was used to perform the statistical analysis. Data are given as mean ± SD. Data comparisons were performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparisons as the post hoc test. P values <0.05 were considered statistically significant.

3. Results

Assessment of the animals' locomotor activity on the rats exposed to Mn caused significant impairment compared to the control group ($P < 0.05$) (Figure 1(a)). It was found that PTX supplementation (300 mg/kg, i.p.) at 5 rpm did not show any significant changes in the animals' locomotor activity in comparison with the Mn receiving group. In the case of stride length evaluation, it was found that both Mn-exposed and PTX-Mn-treated groups reduced the stride length significantly compared to the control group. However, PTX-Mn-treated group showed even more decrease in both left and right hind limb compared to the Mn group although the differences were not significant.

It was found that PTX administration significantly increased the brain tissue biomarkers of oxidative stress (ROS formation, lipid peroxidation). More specifically, ROS was significantly increased in the brain tissue of the Mn group compared to the control group with a P value of less than 0.05 (Figure 2). A clear growth was observed in the ROS formation of the Mn + PTX-treated group compared to the two other tested groups. Similarly, a significant enhancement in lipid peroxidation was found in the Mn + PTX-treated animals ($P < 0.05$).

A significant decrease in the TAC levels was observed in the brain of Mn-exposed rats compared to the control group ($P \leq 0.05$). The Mn + PTX-treated group brought even more decrease in the TAC level compared to both control and Mn-exposed groups ($P \leq 0.05$). As depicted in Figures 3(a) and 3(b), lower brain tissue glutathione level of the Mn + PTX-treated rats was evident in comparison with the Mn and control groups. Administration of PTX appears to deteriorate these parameters.

A significant increase in the Mn plasma level was detected in the animals which received Mn and the Mn + PTX-treated group compared to the control ($P < 0.05$) (Figure 4); however, the statistical analysis did not show any significant difference between the Mn and Mn + PTX-treated groups. Also, it should be noted that administration of PTX increased the Mn concentration in the brain tissue compared to the control group ($P < 0.05$).

4. Discussion

The purpose of this study was to evaluate the possible complications of manganism and PTX. Nowadays, PTX administration has attracted much attention owing to COVID-19, obstructive pulmonary diseases, and peripheral artery diseases due to its anti-inflammatory and smooth muscle relaxation effect [25, 26]. Previous studies have indicated that administration of PTX 400 mg twice a day for 8 weeks effectively controls proinflammatory cytokines [27].

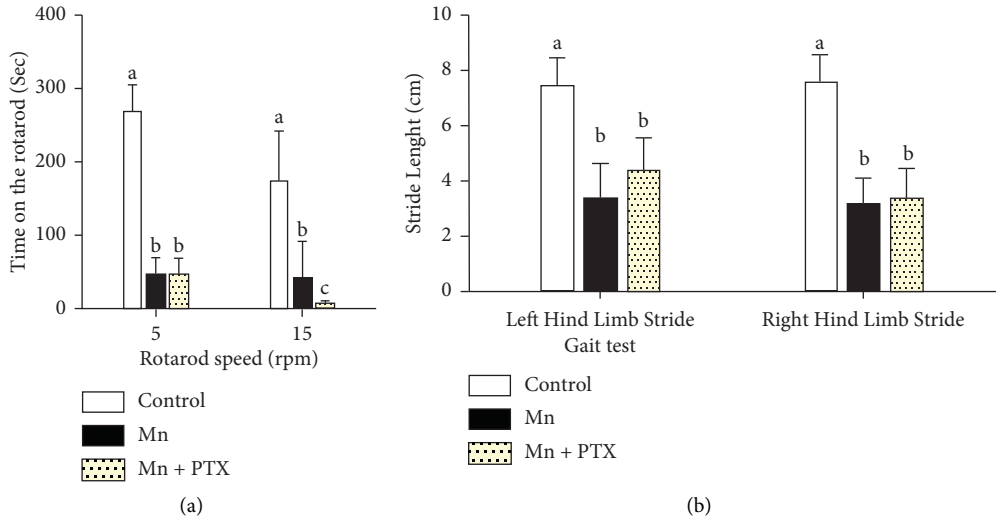

Figure 1: Effect of pentoxifylline supplementation on the animals' locomotor activity in the rat model of manganism. (a) Rotarod test; (b) gait test. Data are represented as mean ± SD. According to the post hoc Tukey test which was used for intergroup comparisons, groups with the same superscripted letters were not significantly different at $\alpha = 0.05$ ($P \geq 0.05$). However, dissimilar letters indicate a significant difference ($P < 0.05$).

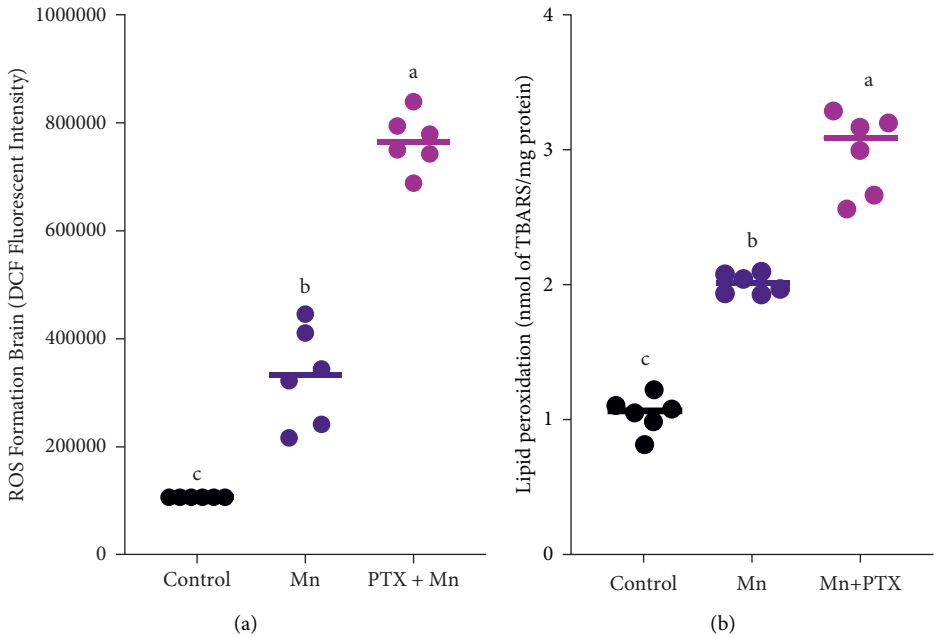

Figure 2: Markers of oxidative stress in the brain of manganese- (Mn-) exposed rats and the effect of PTX treatment. (a) Evaluation of brain tissue ROS formation; (b) assessment of the amount of lipid peroxidation in the brain tissue. Data are represented as mean ± SD. According to the post hoc Tukey test which was used for intergroup comparisons, groups with the same superscripted letters were not significantly different at $\alpha = 0.05$ ($P > 0.05$). However, dissimilar letters indicate a significant difference ($P < 0.05$).

Also, different studies showed that PTX improved the filterability of red blood cells and increased the oxygen delivery to the tissues. PTX was also shown to be effective against ischemic injury of the brain and intestine. PTX is known as an emerging treatment as a vasodilator *via* decreasing the adherence of red blood cells to endothelial cells, blood viscosity, platelet aggregation, and fibrinogen levels [28]. However, drug interactions of PTX with xenobiotic metals in human beings should be more closely monitored.

Industrial sources of xenobiotic metals exposure are a major public health challenge nowadays. Mn is gaining increasing attention because a larger percentage of the population is exposed to industrial pollution, food, air, soil, and water containing a high amount of Mn. Regarding the

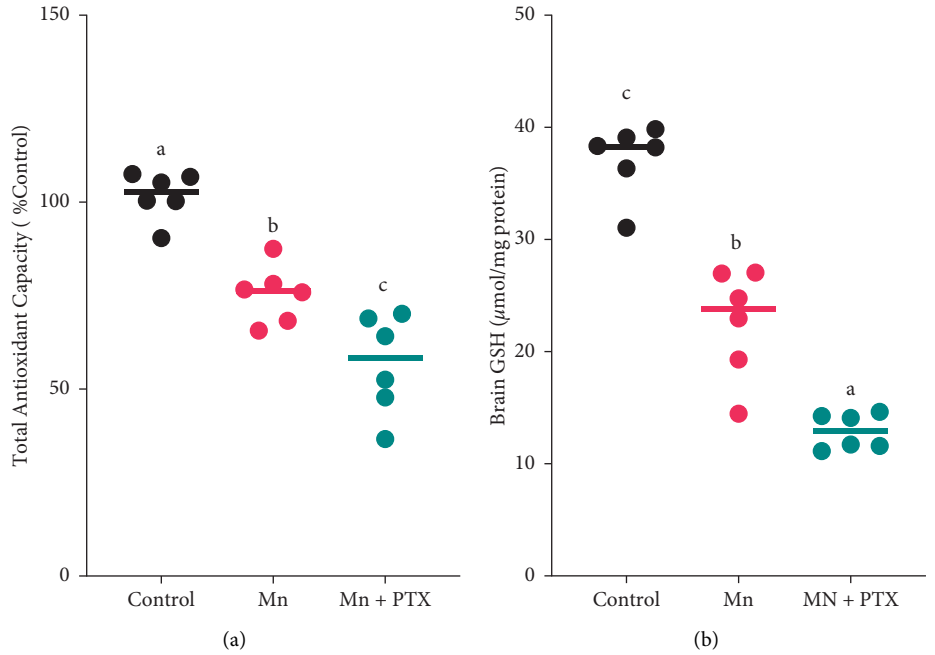

Figure 3: Evaluating the effect of PTX on (a) the total antioxidant capacity (TAC) and (b) glutathione (GSH) content in the brain tissue. Data are represented as mean ± SD. According to the post hoc Tukey test, groups with the same superscripted letters were not significantly different at $\alpha = 0.05$ ($P \geq 0.05$). However, dissimilar letters indicate a significant difference ($P < 0.05$).

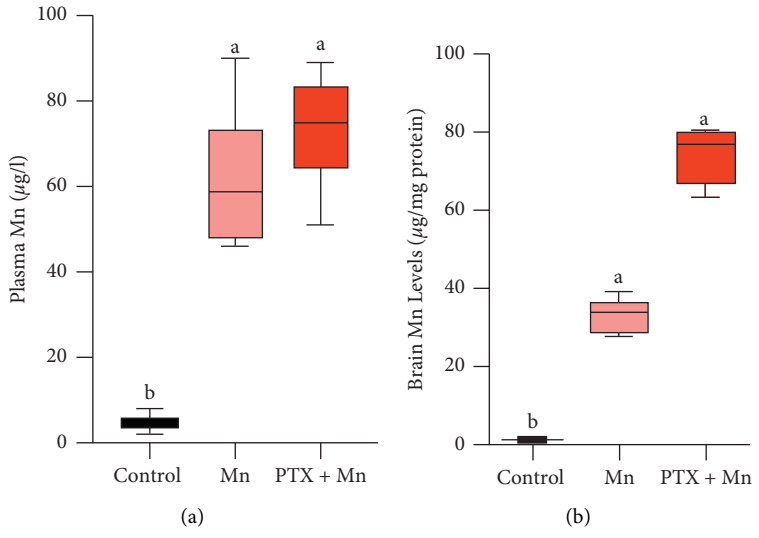

Figure 4: The effects of PTX on the amounts of plasma (a) and brain (b) Mn level in the studied groups. Data are represented as mean ± SD. According to the post hoc Tukey test, groups with the same superscripted letters were not significantly different at $\alpha = 0.05$ ($P \geq 0.05$). However, dissimilar letters indicate a significant difference ($P < 0.05$).

fact that adequate intake levels for Mn for men and women are just 2.3 and 1.8 mg per day, respectively, with the tolerable upper intake level of 11 mg for adults, this has been a cause of some concern due to the high exposure of human to Mn [29, 30]. The routes of Mn exposure are mainly dietary intake, dermal absorption, and inhalation. The distribution of Mn to the body tissues is fairly homogeneous. It was reported that increased concentration of Mn was usually accommodated in tissues rich in mitochondria; therefore, the brain tends to have higher Mn levels. Chronic exposure to this metal can cause a neurodegenerative disease named manganism. A few studies have shown three sites of Mn entry into the brain: the cerebral capillaries, cerebrospinal fluid, and olfactory nerve [30]. Although the mechanisms of Mn toxicity in the brain are not fully elucidated, some evidence indicates that oxidative stress and mitochondria

play major roles in Mn-induced neurotoxicity [31]. The brain is highly susceptible to oxidative stress due to high oxygen consumption, lipid-rich content (phospholipids of cerebral cell membranes), high amounts of prooxidant metal ions, and low activity of the brain antioxidant enzymes [32, 33]. Oxidative stress is associated with increased cell membrane permeability, ATP depletion, and accumulation of abnormal proteins named amyloid β ($A\beta$) and tau [33]. The mentioned process may cause inflammation, neuronal toxicity, and cell death [32]. In addition, astrocytes serve as the major storage site for Mn in the brain and are a prominent contributor to Mn-stimulated nitric oxide (NO) production through inducible nitric oxide synthetase (iNOS). Also, glial cells as an important target of Mn activate the inflammatory signaling pathways which damage the neurons through overproduction of numerous ROS and RNS, as well as the release of IL-6, TNF-α, and the CC chemokines [34, 35].

The correlation between oxidative stress and enhancement of the $A\beta$ deposit in the brain was also reported in different studies. Oxidative stress increases the $A\beta$ peptides (mostly b-sheets structure), oligomers, protofibrils, and fibrils. These peptides subsequently coaggregate with other proteins to produce senile plaques which induce neurodegeneration [36, 37]. On the other hand, $A\beta$ accommodation also exacerbates the oxidative stress conditions by causing mitochondria dysfunction, enhances the ROS, and deteriorates the activity of antioxidant enzymes such as superoxide dismutase (SOD) as glutathione peroxidase (GPx) and catalase (CAT) [38]. Therefore, oxidative stress may play a key role in cerebral inflammation, degeneration, and cognitive impairment [32].

Furthermore, the involvement of oxidative stress in ceramide synthesis has been well documented. Although ceramide is known as a second messenger that regulates the cellular differentiation and proliferation, it has received a lot of attention due to its role as a key mediator in cell death induction and apoptosis [39]. Briefly, ceramide acts directly on the mitochondrial respiratory electron chain and regulation of Mn-SOD program cell death. In addition, ROS production and induced ceramide pathway trigger a neurodegenerative cascade that leads to clinical disease [40].

On the other hand, excessive Mn exposure evokes free radical formation due to the redox-active nature of Mn, resulting in direct oxidation of the membrane lipids, DNA, amino acids, neurotransmitters, and other relevant biomolecules. Also, Mn-induced oxidative stress is caused through mitochondrial dysfunction. Mn^{2+} interferes with Ca^{2+} within the mitochondria by occupying Ca^{2+} binding sites [41, 42], increasing the mitochondrial Ca^{2+} levels, interfering with oxidative respiration, and inducing oxidative stress [43, 44]. Increased intracellular Ca levels may activate the proteases, leading to excitotoxic neuronal death. Several studies have reported that Mn alters the levels of neurotransmitters like glutamate, dopamine, and gamma-aminobutyric acid (GABA). Finally, the approved mechanism for neuronal death of manganism is apoptosis involving DNA cleavage, mitochondrial dysfunction, and proteases activation (like caspases), resulting in cell death [45, 46].

From a toxicology viewpoint, the effect of PTX on the manganism rat model has not been studied yet. As a result, 300 mg of PTX once a day was used to gain insight related to any possible side effects. Our data indicate that PTX on the manganism rat model has an adverse effect through increasing the ROS and lipid peroxidation compared to negative control and even groups receiving Mn . Furthermore, it has been shown that the decline in the TAC and GSH levels correlates with oxidative stress of the tissue and neuroinflammation. Both levels of TAC and GSH were reduced in the brain tissue, demonstrating enhanced toxicity. Our results also showed that PTX increased the risk of Mn accumulation in the brain compared to the control group.

The effect of PTX on biomarkers of oxidative stress, in particular on ROS and lipid peroxidase production, differs from the results previously reported in other studies [47, 48]. It was reported that daily consumption of this drug at 80 mg/kg dose diminished the oxidative damage to the renal tissue induced by streptozotocin in the rat [49]. Reduction of DNA damage was also reported during consumption of 0.5 mm (139 mg/ml) PTX [50]. Recently, it was reported that PTX induced the neuroprotective effect possibly as the result of its anti-inflammatory and TNF-alpha inhibitory properties at 10, 25, and 50 mg/kg doses [41, 42, 51]. In line with previous investigations, Mn significantly reduced the antioxidant enzyme such as TAC and GSH (observed in the Mn-receiving group) and increased the ROS and lipid peroxidation compared to the control group [52]. In other studies, it was shown that excessive Mn exposure leads to neurological complications, oxidative stress, and mitochondrial injury. Assessments of taurine on the manganism model confirmed its neuroprotective properties through alleviated Mn-induced locomotor deficit, relieved the oxidative stress biomarkers, and preserved the brain tissue mitochondrial indices of functionality [17]. A similar study showed that locomotor dysfunction, brain tissue biomarkers of oxidative stress, and impairment of mitochondrial indices were seen in the Mn-exposed animals. It was found that carnosine supplementation abrogated the biomarkers of oxidative stress in the brain tissue and improved the brain mitochondrial function of Mn-exposed animals [18]. It seems that carnosine is a potential neuroprotective agent against Mn neurotoxicity. Also, it was found that the toxicity in mice with manganism was alleviated by herbal formula B401 (50 mg/kg) treatment. The herbal formula B401 contains six herbal ingredients: extracts from *Panax ginseng*, *Astragalus membranaceus*, *Angelica sinensis*, *Rehmannia glutinosa*, *Ligustri Fructus*, and *Eclipta prostrata*, which enhance both nitric oxide synthesis and angiogenesis. B401 also reduced the oxidative stress, inflammation, and apoptosis in penile corpus cavernosum [53]. In another study, the effectiveness of trehalose to interfere with the Mn-induced mitochondrial dysfunction in a mouse model of manganism was explored. Biological results indicated that trehalose pretreatment significantly reduced oxidative damage and enhanced the activation of mitophagy [54]. Additionally, pretreatment with vitamin E (100 mg/kg, i.p.), as an antioxidant, or ibuprofen (140 μg/mL in the drinking water for two weeks)

as anti-inflammatory agent attenuated the increase in cerebral F2-isoprostanes (F2-IsoPs) and protected the striatal medium spiny neurons from dendritic atrophy and dendritic spine loss [55].

An interesting finding in our study was that PTX administration (300 mg/kg for 8 consecutive days) increased the manganism complications in the rat. Given that the PTX therapeutic dose is 400 mg twice a day, our preliminary results support the view that PXT consumption may be harmful if administered in manganism- and metal-poisoned patients. We propose that PXT possesses significant blood thinning and vasodilator properties as well as deformability and rheological changes of red blood cells (confirmed by atomic force microscopy nanoindentation experiment) [56, 57]. PTX decreases the blood and plasma viscosity and lowers plasma fibrinogen while promoting fibrinolysis; it also improves blood filterability by enhancing the erythrocyte distensibility and lessens neutrophil activation [58]. Moreover, PTX improves the tissue perfusion through small capillaries by improving the deformability of red blood cells. According to the present study, PTX at the high dose may increase the chance of Mn to pass through BBB and reach the site of action to induce manganism. Industrial occupations, especially miners, welders, and smelters, are more exposed to metal toxic substances, and consumption of blood thinner may lead to negative health effects. From a broader perspective, it can be generalized that blood-thinning drugs at high doses (in this case, PTX) may exacerbate the complications of manganism and brain metal toxicity.

The main limitation of the current study is that the exact mechanisms that explain that PTX evokes Mn toxicity are lacking to correctly interpret the results of animal models into clinical practice. In addition, a limited number of oxidative stress factors were evaluated in the present study. In a future project, measuring other factors such as 8-hydroxy-2-deoxyguanosine, tissue lipid peroxidation, mitochondrial swelling, and mitochondrial ATP level will give a more complete picture of how PTX at a high dose responds to increased oxidative stress. Also, histopathological evaluation of the brain tissue could clarify the tissue injury during consumption of PTX during exposure to heavy and toxic metals.

5. Conclusions

The main findings of this study provided new insights into the consumption of PTX that at a high dose may contraindicate with Mn exposure and manganism in humans, especially in industrial workers. The data provided in the current study indicate that PTX at high doses stimulates ROS and oxidative stress which leads to dysfunction of the brain tissue. Also, PTX administration induced oxidative damage *via* significant reduction of TAC and GSH content as well as enhanced levels of Mn in the brain tissue compared to the control group.

According to our preliminary study, it is better to limit the administration of the maximum dose of PTX in manganism disorders. However, further studies are warranted to confirm the clinical significance of these data.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. All data are presented in the form of tables and figures.

Disclosure

Shiraz University of Medical Sciences was not involved in the design of the study and collection, analysis, and interpretation of data as well as in writing the manuscript. This study was part of the Pharm.D thesis of Romina Tanideh.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding this work.

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References

[1] S. Bouabid, A. Tinakoua, N. Lakhdar-Ghazal, and A. Benazzouz, "Manganese neurotoxicity: behavioral disorders associated with dysfunctions in the basal ganglia and neurochemical transmission," *Journal of Neurochemistry*, vol. 136, no. 4, pp. 677–691, 2016.

[2] M. Aschner, "Manganese: brain transport and emerging research needs," *Environmental Health Perspectives*, vol. 108, no. 3, pp. 429–432, 2000.

[3] P. Chen, N. Parmalee, and M. Aschner, "Genetic factors and manganese-induced neurotoxicity," *Frontiers in Genetics*, vol. 5, no. 265, 2014.

[4] P. Karki, E. Lee, and M. Aschner, "Manganese neurotoxicity: a focus on glutamate transporters," *Annals of Occupational and Environmental Medicine*, vol. 25, no. 1, p. 4, 2013.

[5] U. Förstner and G. T. Wittmann, *Metal Pollution in the Aquatic Environment*, Springer Science & Business Media, Berlin, Germany, 2012.

[6] J. Crossgrove and W. Zheng, "Manganese toxicity upon overexposure," *NMR in Biomedicine*, vol. 17, no. 8, pp. 544–553, 2004.

[7] G. R. Evans and L. N. Masullo, *Manganese Toxicity*, StatPearls, Treasure Island, FL, USA, 2020.

[8] S. Caito and M. Aschner, "Neurotoxicity of metals," in *Handbook of Clinical Neurology*, M. Lotti and M. L. Bleecker, Eds., Elsevier, Amsterdam, The Netherlands, pp. 169–189, 2015.

[9] Y. Kim, J.-M. Kim, J.-W. Kim et al., "Dopamine transporter density is decreased in parkinsonian patients with a history of manganese exposure: what does it mean?" *Movement Disorders*, vol. 17, no. 3, pp. 568–575, 2002.

[10] T. V. Peres, M. R. C. Schettinger, P. Chen et al., "Manganese-induced neurotoxicity: a review of its behavioral consequences and neuroprotective strategies," *BMC Pharmacology and Toxicology*, vol. 17, no. 1, pp. 1–20, 2016.

[11] J. E. Frampton and R. N. Brogden, "Pentoxifylline (oxpentifylline)," *Drugs & Aging*, vol. 7, no. 6, pp. 480–503, 1995.

[12] A. Ward and S. P. Clissold, "Pentoxifylline," *Drugs*, vol. 34, no. 1, pp. 50–97, 1987.

[13] G. M. Doherty, J. C. Jensen, H. R. Alexander, C. M. Buresh, and J. A. Norton, "Pentoxifylline suppression of tumor necrosis factor gene transcription," *Surgery*, vol. 110, no. 2, pp. 192–198, 1991.

[14] J. L. Fernandes, R. T. D. de Oliveira, R. L. Mamoni et al., "Pentoxifylline reduces pro-inflammatory and increases anti-inflammatory activity in patients with coronary artery disease-A randomized placebo-controlled study," *Atherosclerosis*, vol. 196, no. 1, pp. 434–442, 2008.

[15] D. Brie, A. Sahebkar, P. E. Penson et al., "Effects of pentoxifylline on inflammatory markers and blood pressure," *Journal of Hypertension*, vol. 34, no. 12, pp. 2318–2329, 2016.

[16] C. A. Dodd, D. L. Ward, and B. G. Klein, "Basal Ganglia accumulation and motor assessment following manganese chloride exposure in the C57BL/6 mouse," *International Journal of Toxicology*, vol. 24, no. 6, pp. 389–397, 2005.

[17] M. M. Ommati, R. Heidari, V. Ghanbarinejad, N. Abdoli, and H. Niknahad, "Taurine treatment provides neuroprotection in a mouse model of manganism," *Biological Trace Element Research*, vol. 190, no. 2, pp. 384–395, 2019.

[18] M. M. Ommati, R. Heidari, V. Ghanbarinejad, A. Aminian, N. Abdoli, and H. Niknahad, "The neuroprotective properties of carnosine in a mouse model of manganism is mediated via mitochondria regulating and antioxidative mechanisms," *Nutritional Neuroscience*, vol. 23, no. 9, pp. 731–743, 2020.

[19] R. J. Carter, J. Morton, and S. B. Dunnett, "Motor coordination and balance in rodents," *Current Protocols in Neuroscience*, vol. 15, no. 1, pp. 8.12.1–8.12.14, 2001.

[20] M. M. Ommati, A. Jamshidzadeh, H. Niknahad et al., "*N*-acetylcysteine treatment blunts liver failure-associated impairment of locomotor activity," *PharmaNutrition*, vol. 5, no. 4, pp. 141–147, 2017.

[21] R. Heidari, H. Babaei, L. Roshangar, and M. A. Eghbal, "Effects of enzyme induction and/or glutathione depletion on methimazole-induced hepatotoxicity in mice and the protective role of *N*-acetylcysteine," *Advanced Pharmaceutical Bulletin*, vol. 4, no. 1, pp. 21–8, 2014.

[22] R. Gupta, D. Dubey, G. Kannan, and S. Flora, "Concomitant administration of Moringa oleifera seed powder in the remediation of arsenic-induced oxidative stress in mouse," *Cell Biology International*, vol. 31, no. 1, pp. 44–56, 2007.

[23] D. J. Socci, K. B. Bjugstad, H. C. Jones, J. V. Pattisapu, and G. W. Arendash, "Evidence that oxidative stress is associated with the pathophysiology of inherited hydrocephalus in the *H*-Tx rat model," *Experimental Neurology*, vol. 155, no. 1, pp. 109–117, 1999.

[24] J. Sedlak and R. H. Lindsay, "Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent," *Analytical Biochemistry*, vol. 25, pp. 192–205, 1968.

[25] F. Seirafianpour, S. Mozafarpoor, N. Fattahi, A. Sadeghzadeh-Bazargan, M. Hanifiha, and A. Goodarzi, "Treatment of COVID-19 with pentoxifylline: could it be a potential adjuvant therapy?" *Dermatologic Therapy*, vol. 33, no. 4, Article ID e13733, 2020.

[26] M. Ghasemnejad-Berenji, S. Pashapour, and S. Sadeghpour, "Pentoxifylline: a drug with antiviral and anti-inflammatory effects to be considered in the treatment of coronavirus disease 2019," *Medical Principles and Practice*, vol. 30, no. 1, pp. 98–100, 2021.

[27] G. Gutierrez-Reyes, P. Lopez-Ortal, S. Sixtos et al., "Effect of pentoxifylline on levels of pro-inflammatory cytokines during chronic hepatitis *C*," *Scandinavian Journal of Immunology*, vol. 63, no. 6, pp. 461–467, 2006.

[28] H. R. D. Dolatabadi, M. R. Zarrindast, P. Reisi, and M. Nasehi, "The effects of pentoxifylline on serum levels of interleukin 10 and interferon gamma and memory function in lipopolysaccharide-induced inflammation in rats," *Advanced Biomedical Research*, vol. 6, p. 110, 2017.

[29] R. Russell, J. L. Beard, R. J. Cousins et al., Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc, a report of the panel on micronutrients, subcommittees on upper reference levels of nutrients and of interpretation and uses of dietary reference intakes, and the standing committee on the scientific evaluation of dietary reference intakes food and nutrition board institute of medicine, 2001.

[30] A. W. Dobson, K. M. Erikson, and M. Aschner, "Manganese neurotoxicity," *Annals of the New York Academy of Sciences*, vol. 1012, no. 1, pp. 115–128, 2004.

[31] M. Saeedi, D. Mohtadi-Haghighi, S. S. Mirfazli et al., "Design and synthesis of selective acetylcholinesterase inhibitors: arylisoxazole-phenylpiperazine derivatives," *Chemistry and Biodiversity*, vol. 16, no. 2, Article ID e1800433, 2019.

[32] S. Salim, "Oxidative stress and the central nervous system," *Journal of Pharmacology and Experimental Therapeutics*, vol. 360, no. 1, pp. 201–205, 2017.

[33] M. Maciejczyk, E. Żebrowska, and A. Chabowski, "Insulin resistance and oxidative stress in the brain: what's new?" *International Journal of Molecular Sciences*, vol. 20, no. 4, Article ID 874, 2019.

[34] K. S. Kirkley, K. A. Popichak, M. F. Afzali, M. E. Legare, and R. B. Tjalkens, "Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity," *Journal of Neuroinflammation*, vol. 14, no. 1, pp. 99–18, 2017.

[35] R. B. Tjalkens, K. A. Popichak, and K. A. Kirkley, "Inflammatory activation of microglia and astrocytes in manganese neurotoxicity," *Advances in Neurobiology*, vol. 18, pp. 159–181, 2017.

[36] K. Chen, M. Kazachkov, and P. H. Yu, "Effect of aldehydes derived from oxidative deamination and oxidative stress on β-amyloid aggregation; pathological implications to Alzheimer's disease," *Journal of Neural Transmission*, vol. 114, no. 6, pp. 835–839, 2007.

[37] G. Merlini and V. Bellotti, "Molecular mechanisms of amyloidosis," *New England Journal of Medicine*, vol. 349, no. 6, pp. 583–596, 2003.

[38] T. C. Squier, "Oxidative stress and protein aggregation during biological aging," *Experimental Gerontology*, vol. 36, no. 9, pp. 1539–1550, 2001.

[39] N. Andrieu-Abadie, V. Gouazé, R. Salvayre, and T. Levade, "Ceramide in apoptosis signaling: relationship with oxidative stress," *Free Radical Biology and Medicine*, vol. 31, no. 6, pp. 717–728, 2001.

[40] R. G. Cutler, J. Kelly, K. Storie et al., "Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease," *Proceedings of the National Academy of Sciences*, vol. 101, no. 7, pp. 2070–2075, 2004.

[41] K. R. Neves, H. V. Nobre, L. K. Leal, G. M. de Andrade, G. A. Brito, and G. S. Viana, "Pentoxifylline neuroprotective effects are possibly related to its anti-inflammatory and TNF-alpha inhibitory properties, in the 6-OHDA model of Parkinson's disease," *Parkinson's Disease'*, vol. 2015, Article ID 108179, 2015.

[42] S. M. Evans, L. M. Pinto Pereira, and J. I. Addae, "Neuroprotection by caffeine and pentoxifylline during experimental cerebral ischaemia," *West Indian Medical Journal*, vol. 48, no. 1, pp. 23–25, 1999.

[43] O. M. Ijomone, O. M. Aluko, C. O. A. Okoh, A. C. Martins Jr, and M. Aschner, "Role for calcium signaling in manganese neurotoxicity," *Journal of Trace Elements in Medicine & Biology*, vol. 56, pp. 146–155, 2019.

[44] A. C. Martins, P. Morcillo, O. M. Ijomone et al., "New insights on the role of manganese in Alzheimer's disease and Parkinson's disease," *International Journal of Environmental Research and Public Health*, vol. 16, no. 19, Article ID 3546, 2019.

[45] J. A. Roth, "Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination," *Biological Research*, vol. 39, no. 1, pp. 45–57, 2006.

[46] L. Quintanar, "Manganese neurotoxicity: a bioinorganic chemist's perspective," *Inorganica Chimica Acta*, vol. 361, no. 4, pp. 875–884, 2008.

[47] X. Zhang, R. K. Sharma, A. Agarwal, and T. Falcone, "Effect of pentoxifylline in reducing oxidative stress-induced embryotoxicity," *Journal of Assisted Reproduction and Genetics*, vol. 22, no. 11, pp. 415–417, 2005.

[48] K. A. McKinney, S. E. Lewis, and W. Thompson, "The effects of pentoxifylline on the generation of reactive oxygen species and lipid peroxidation in human spermatozoa," *Andrologia*, vol. 28, no. 1, pp. 15–20, 1996.

[49] M. E. Dávila-Esqueda and F. Martínez-Morales, "Pentoxifylline diminishes the oxidative damage to renal tissue induced by streptozotocin in the rat," *Experimental Diabesity Research*, vol. 5, no. 4, pp. 245–251, 2004.

[50] C. Laurent, P. Voisin, and J.-P. Pouget, "DNA damage in cultured skin microvascular endothelial cells exposed to gamma rays and treated by the combination pentoxifylline and α-tocopherol," *International Journal of Radiation Biology*, vol. 82, no. 5, pp. 309–321, 2006.

[51] B.-L. Eun, X.-H. Liu, and J. D. E. Barks, "Pentoxifylline attenuates hypoxic-ischemic brain injury in immature rats," *Pediatric Research*, vol. 47, no. 1, p. 73, 2000.

[52] J. O. Ciocon, D. Galindo-Ciocon, D. J. Galindo, and J. O. Ciocon, "A comparison between aspirin and pentoxifylline in relieving claudication due to peripheral vascular disease in the elderly," *Angiology*, vol. 48, no. 3, pp. 237–240, 1997.

[53] C. H. Hsu, C. L. Lin, S. E. Wang, S. J. Sheu, C. T. Chien, and C. H. Wu, "Oral treatment with herbal formula B401 alleviates penile toxicity in aging mice with manganism," *Clinical Interventions in Aging*, vol. 10, pp. 907–918, 2015.

[54] K. Liu, M. J. Jing, C. Liu et al., "Effect of trehalose on manganese-induced mitochondrial dysfunction and neuronal cell damage in mice," *Basic and Clinical Pharmacology and Toxicology*, vol. 125, no. 6, pp. 536–547, 2019.

[55] D. Milatovic, R. C. Gupta, Y. Yu, S. Zaja-Milatovic, and M. Aschner, "Protective effects of antioxidants and anti-inflammatory agents against manganese-induced oxidative damage and neuronal injury," *Toxicology and Applied Pharmacology*, vol. 256, no. 3, pp. 219–226, 2011.

[56] I. Hassan, K. Dorjay, and P. Anwar, "Pentoxifylline and its applications in dermatology," *Indian Dermatology Online Journal*, vol. 5, no. 4, pp. 510–516, 2014.

[57] K. E. Aifantis, S. Shrivastava, S.-H. Pelidou, A. H. W. Ngan, and S. I. Baloyannis, "Relating the blood-thinning effect of pentoxifylline to the reduction in the elastic modulus of human red blood cells: anin vivo study," *Biomaterials science*, vol. 7, no. 6, pp. 2545–2551, 2019.

[58] M. F. McCarty, J. H. O'Keefe, and J. J. DiNicolantonio, "Pentoxifylline for vascular health: a brief review of the literature," *Open Heart*, vol. 3, no. 1, Article ID e000365, 2016.