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AMELIORATIVE EFFECTS OF *MENTHA SPICATA* ESSENTIAL OIL ON LEAD AND MANGANESE-INDUCED BRAIN OXIDATIVE DAMAGE IN RATS

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ABSTRACT

This study evaluated the antioxidant activity of *Mentha Spicata* essential oil against to lead and manganese-induced brain oxidative damage in Wistar rats. Chronic oral co-exposure to lead (0.2%) and manganese (4.79 mg/ml) during periods of gestation and lactation resulted in a significant reduction in both body and brain weight. produced significantly reduced in superoxide dismutase (SOD) content, and decreased catalase (CAT), and glutathion-peroxydase (GPx) activities were observed in the braintissues; however, the administration of the essential oil of *Mentha spicata* with intraperitoneal injection of 0.1 ml HEM/kg over a 21-days periodsignificantly improved the various in the both body and brain weight. These results indicate that *Mentha spicata* has an antioxidant effect against lead and manganese-induced brain oxidative damage and is useful as a neuroprotective agent against various brain diseases induced by oxidative stress.

Keywords: Lead, Manganese, *Mentha Spicata*, Oxidative stress, Brain.

1. INTRODUCTION

In recent years, lead has become a regulatory preoccupation and the focus of much interest among pharmacologists, environmental scientists and clinicians because of its persistence in human and animal tissues, which has often been associated with significant health risks1.

In addition, manganese is a ubiquitous element in human and animal life and is an important factor in brain development and function as a cofactor of several enzymes involved in the synthesis and metabolism of neurotransmitters, including glutamine synthetase, arginase[2], pyruvate decarboxylase and mitochondrial superoxide dismutase[3].

In fact, the various studies undertaken in rats and humans have shown that lead is toxic even at relatively low doses, whereas manganese at high doses has deleterious effects. These two elements affect all compartments of the living organism and the most vulnerable organs, essentially the neurological system[4].

For a long time, mint (*Mentha Spicata*) has been used as powerful fungicides, pesticides, and insecticides, hypotensive, antiposmodic, anti-oxidants, anti-inflammatory, hepatoprotective and anti-microbial[5].

Accordingly, the present study examined the putative antioxidant activity of *Mentha Spicata* vis-à-vis the chronic lead and manganese-induced brain oxidative damage in wistar rats during the period of development (gestation and lactation).

2. MATERIALS AND METHODS

2.1. Extraction of essential oil

The leaves of spearmint (*Mentha spicata*) were harvested in SidiMaàmarwilaya of Saida in the western Algerian highlands, then identified by taxonomic experts (PrHasnaoui and Drsitayeb) University of Saida – Algeria. The essential oil of mint (*Mentha spicata*) was extracted by hydrodistillation which is based on the use of a quantity of 50g of the leaves of mint with 500 ml of distilled water[6]

2.2. Distribution of lots

The experiments were carried out on Wistar rats, which weighed between 200 and 400 g. The rats are grouped in cages at a rate of 2 females and one male. They are placed in a ventilated animal house, with a temperature of 21 ◦C ± 1 ◦C with lighting artificial which establishes a day/night cycle (day between 7 and 19 h). On the first day of gestation, females are divided into two groups:

The intoxication of females begins on the first day of cohabitation with male rats which is represented by D0 and continues during the gestation period. Newborns are also exposed to Pb-Mn until weaning (21 days after birth). Progeny are subject to the same experimental condition.

Group Pb-Mn

consisting of animals which receive orally lead acetate (Pb(C2H3O2)2) at 0.2% and manganese chloride tetrahydrate (MnCl24H2O) at 4.79 mg/ml in bidistilled water orally from the first day of gestation until weaning (n = 07 male rats).[7,8]

Group T is the control batch (T) that receives the distilled water. Tested offspring are subject to the same conditions as their mother.

Group T-MEO

24 hours after weaning, animals receiving distilled water are treated with HEM mint essential oil (0.1 ml/kg) with one intraperitoneal injection per day for 21 days (n = 07 male rats).

Group Pb-Mn-MEO

24 hours after weaning, animals receiving Pb-Mn are treated with HEM mint essential oil (0.1 ml/kg) with one intraperitoneal injection per day for 21 days (n = 07 male rats).

2.3. Body and Brain weight

The body weight of each animal was daily recorded throughout the duration of the experiment. The left-brain weights of different groups of animals were registered.

2.4. Biochemical tests

Measuring the activity of antioxidant enzymes

The Brain of the rats was weighed and homogenized in a buffer solution containing 0.32 M sucrose, 0.5 mM EDTA, 10mM Tris-Hcl (pH 7.4) in ice (1mg tissue / 4ml buffer solution), using a glass tissue homogenizer. The tissue was kept at a temperature of 4°C throughout the dissection and homogenization procedures. The homogenate was centrifuged at 1000xg for 15 minutes at 4°C. The resulting supernatant was centrifuged at 1000xg for 15 minutes at 4°C. The pellet consisted of the mitochondrial fraction, and the supernatant was re-centrifuged at 10,000xg/30 minutes. The two resultant pellets were made soluble in a buffer solution containing 0.32 M sucrose, 0.5 mM EDTA, 10 mMTris-Hcl and 0.02% digitonin (pH 7.4). The digitonin was added to liberate any mitochondria entrapped in the synaptosomes, and centrifuged a second time at 10,000xg for 15 minutes at 4°C. The resultant pellet consisted of the total mitochondrial fraction, which was made soluble in a solution containing sucrose (0.32 M at pH 7.4)[9]. The superoxide dismutase (SOD) (EC 1.15.1.1) was analyzed in the supernatant using the technique of Kakkar (1984)[10]; this method is based on inhibiting the formation of nicotinamide adenine dinucleotide, phenazine methosulfate and formazan blue tetrazolium. The activities and antioxidant levels in the kidneys, as well as those of catalase (CAT) and glutathione peroxidase (Gpx) were analyzed using the methods of Sinha, (1972)[11] and Rotruck ,(1973)[9], respectively.

2.5. Statistical expression and analysis of the results

The results are expressed as the mean (M) of the individual values, subject to standard error of the mean (SEM). Multiple means are compared through analysis of variance (ANOVA) with the intoxication factor (Pb-Mn, T). ANOVAs with repeated measures were used to analyze the time factor. A probability of $p < 0.05$ is considered significant. Statistical analyses

were performed using the Sigma Stat software (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. The yield of essential oil

The hydro-distillation of the plant matter *Mentha spicata* allowed to obtain a yield of 0.49%.

3.2. Effects on the body and the brain weight

The results show that the animals exposed to Pb-Mn presented a significant reduction ($p < 0.01$) in body weight compared to that of the control animals during the experiment. Additionally, the animals exposed to Pb-Mn and treated with MEO showed a significant increase ($p < 0.001$) in body weight in comparison with the untreated rats (Table 01).

The results found in the poisoned animals also reveal a significant decrease ($p < 0.01$) in brain weight compared to the control group (Table 01). On the other hand, the animals treated with MEO showed a significant increase ($p < 0.01$) in the weight of the studied organ in comparison with the untreated rats).

3.3. Effect of lead and manganese and HEM on the enzymatic activity of oxidative status in the brain

The brain antioxidant results show a significant decrease in SOD, CAT and GPx in the brain of the rats exposed to Pb-Mn during the developmental period in comparison with the control rats. Under MEO treatment, on the other hand, a significant increase was observed ($p \leq 0.05$) in the group treated following poisoning (Table 02).

4. DISCUSSION

The *Mentha Spicata* essential oil has been obtained by hydro-distillation with a productivity of 0.49%, this disagrees with the works of Priscilla et al.[6] who reported an estimation of (0,23±0,04%) and with those of Lucchesiet al.[12] (0,60±0,06-%). This difference in productivity can be attributed to many factors which are mainly the origin, the species, the harvest period, the duration of drying and the technique of essential oil extraction[13].

The results recorded clearly show that the weight of the animals exposed to Pb-Mn during the prenatal period was significantly lower than that of the control animals, which manifested as a decrease in body weight gain. This can be explained by the anorectic effect of Pb-Mn and their impact on the nervous centers responsible for the regulation of satiety and hunger. Our results are in keeping with the work undertaken by various authors Ibrahim et al.[14] and Torres et al.[15]. They observed a reduction in the food consumption of poisoned rats depending on the dose administered and the duration of exposure. Furthermore, we have observed that the administration of lead acetate leads to a reduction in Brain weight, which suggests disruption to their function and which may be due to the effect of Pb and Mn on neuronal proliferation and differentiation in the course of the prenatal period[16;17].

Consequently, the administration of the essential oil of *Mentha spicata* to rats previously exposed to Pb-Mn led to the observation of a clear increase in body weight gain in comparison with the animals left untreated following exposure to Pb-Mn. This recorded increase in weight could be due to the presence of terpenoid compounds, which act to stimulate the transport of glucose in cells[18]. Our observations are also in keeping with those of Toghyani*et* al.[19], who reported that the addition of peppermint powder to the diet of table chickens at a level of 4 g/kg led to an increase in body weight gain. The same has also been shown by Zargari et al.[20], that the addition of medicinal plants stimulates appetite and the secretion of gastrointestinal fluids, and improves digestion and absorption, thus resulting in body weight gain.

Effects of lead and manganese and MEO on oxidative stress in the brain

Exploration of antioxidant enzymes in rats Co-exposed by lead and manganese revealed considerable perturbations in the activity of the different enzymes (CAT, GPx, SOD) in the intoxicated group compared to the control rats at the brain level.

The oral administration of lead and manganese to rats causes a very significantdecrease in SOD and GPx activity in the brain. These results show that exposure to lead over a relatively long period of time induces the reduction of enzyme activity responsible for defenses against the production of free radicals in the body at the brain level[21]**.** The antioxidant activity of superoxide dismutase (SOD) decreased in all regions of the brain after lead exposure[22]**.**

In addition, Pb depletes major antioxidant cells, particularly enzymes that contain the thiol group (e. g. glutathione)[23]**.** Moreover, exposure to Pb causes a significant decrease in the activity of mitochondrial complex I-III / II-III in Hc/St and decreases total thiol levels[24]**.**

Other studies by Annabiet al.[23] have also observed alterations in the activity of certain enzymes in the redox system in the brains of Pb intoxicated rats. The consequence of the decrease in antioxidants on tissues is due to the increase in the peroxidation rate of lipids, modified calcium and sulfhydrile homeostasis. All these processes can be associated with levels of enhanced ROS in the cell, followed by oxidative stress.

Our results are in accordance with Moreira et al.[21], which reveal a significant decrease in SOD activity of lead-exposed rats. However, some researchers have evaluated the activity of antioxidant enzymes in brain regions instead of the entire brain because different regions may react differently to oxidative stress[22]**.**

In more, the lead does not undergo an Oxido-reducing cycle, so this peroxidation is indirectly due to its effect on GPx and other antioxidant enzymes, attributed to the high affinity of lead for sulfhydril groups and metal cofactors present in these enzymes. Lead also acts by disrupting mitochondrial functions through the elevation of [Ca2+], all of which contributes to stimulating the synthesis of free radicals. The peroxidation of lipids that follows causes the breakage of polyunsaturated fatty acid chains and thus the end of the selective permeability of the membrane, resulting in the swelling and necrosis of neural and glial cells[25].

However, these results are in harmony with those of latronicoet al.[26] who determined the intracellular redox state of manganese-treated astrocytes, the evaluation of superoxide dismutase (SOD) activity and reactive oxygenated species (ROS) recorded a significant increase in ROS and a decrease in superoxide dismutase (SOD) activity.

In the same context, Bhuvaneswari et al.[27] show that exposure to low or high-dose Mn causes oxidative damage in different regions of the brain (cerebral cortex,cerebellum and hippocampus) leads to altered activity of the enzymes SOD, CAT, GPx isoforms, and the genes for Mn-SOD and GPx expression.

It is suggested that the first step in the production of ROS is the production of O_2- which can be converted to H_2O_2 by Mn and Cu/Zn superoxide dismutase in mitochondria and cytoplasm. H_2O_2 can still be converted to OH. In the presence of Mn or other transition metals[28]**.**

In the same way, the administration of MEO by intraperitoneal at a dose of 0.1ml/kg showed a significant increase in SOD content and GPx activity in rats Co-exposed to Pb and Mn. Our results are in accord with Hassan et al.[29], which indicate that the gavage of *Menthapiperita* extract caused a significant increase in SOD content and GPx activity in rats previously having oxidative stress induced by gamma irradiation.

Brahmi et al.[30], demonstrate that *Mentha* leaf extract has been shown to have antioxidant, anti-peroxidant and antimutagenic properties.

Some studies report that this extract from the *Mentha* leaf provide protection against radiation-induced cellular damage through delay or prevent the occurrence of radiation-induced oxidative stress [31]**.**

However, pre-treatment for mice irradiated by gamma rays with Menthapiperita extract (1g / 1Kg/body-weight/day) significantly eliminated the radiation-induced increases in NO, H2O2, K and Fe levels, as well as significantly improved GSH, Gpx, SOD, AchE[29]**.**

CONCLUSION

The exposure of wistar rats, during gestation and lactation, to Pb-Mn revealed a dysfunction in the anti-oxidative defense system, which results in an important alteration in the anti-radical system represented by the different enzymes. The treatment with MEO in previously intoxicated rats leads to a rehabilitation of this system and a rectification of this disorders.

Table 01: Effect of *M. spicata* oil on the body and the brain weights in ratsintoxicated (Pb-Mn)

Groups (g)	Control	HEM	Pb-Mn	Pb-Mn-HEM
Bodyweight(g)	95,03 ±1,70	100,56±0,7	67,88±1,35*	76,17 ±1,28*
Brain weight (g)	01,49±0,033	01.50 ±0,031	1.23±0,026*	1.39±0,028*

The values are expressed as an average ± SEM (*: p<0.05)

Table 02: Antioxidant enzyme activity (SOD, CAT, GPx) in the brain of intoxicated and intoxicated rats treated with MEO, controls and treatments

	Pb-Mn	Pb-Mn+ MEO	Control	MEO
SOD (U/mg of protein)	1.18± 0,48*	1.54±0,73*	1.61±1,85	1.58±0,51
CAT (U/mg of protein)	0.41±1,04*	0.54±0,77*	0.68± 0,58	0.64±0,7
GPx(U/mg of protein)	0.022±0,59*	0.035±0,52*	0.041±0,067	0.039±0,02

The values are expressed as an average ± SEM (*: p<0.05).

REFERENCES

1. Tajudeen O, Amos O, Akinwunmi A, John AF, David JD, Ehizode FI, Adeleke O, Adewale F, Olakunle BA, Joseph O and Benjamin O. Protective effect of methanolic and flavonoid-rich leaf extracts of Synsepalumdulcificum (Danielli) on lead-acetate-induced toxicity in Wistar albino rats. Journal of Applied Pharmaceutical Science. 2019;9(05):065-072.
2. Shishova EY, Di Costanzo L, Emig FA, Ash DE and Christianson DW. Probing the specificity determinants of amino acid recognition by arginase. Biochemistry. 2009;48:121–131.
3. Bouabid S, Tinakoua A, Lakhdar-ghazel N and Benazzouz A. Manganese Neurotoxicity: behavioral disorders associated with dysfunctions in the basal ganglia and neurochemical transmission. Journal of neurochemistry. 2016;136:677-691.
4. Racette BA, Aschner M, Guilarte TR, Dydak U, Criswell SR and Zheng W. Pathophysiology of manganese-associated neurotoxicity. Neurotoxicology. 2012;33(4):881-886.
5. Benomari FZ, Andreu V, Kotarba J, Dib MA, Bertrand C, Muselli A, Costa J and Djabou N. Essential oils from Algerian species of Mentha as new bio-control agents against phytopathogen strains. Environmental Science and Pollution Research. 2017;1-12.
6. Priscilla PA, Natália M and Sandra FRS. Extraction of Men-thaspicata L. Volatile Compounds: Evaluation of Process Parame-ters and Extract Composition. Food and Bioprocess Technology. 2010;3(4):1-12.
7. Kahloula K, Slimani M, Dubois M and Bonnet JJ. D-cyclo serine enhances spatial learning performances of rats chronically ex-posed to lead during the developmental period. African Journal of Neurological Sciences.2009;28:67-77.
8. Brahmi M, Adli DEH, Slimani M, Arabi W, Taibi N and Kahloula K. Evaluation of the therapeutic effects of Mentha spicata essential oil at the liver level in developing wistar rats co-exposed to lead and manganese. Carpathian Journal of Food Science and Technology. 2019;11(2):152-164.
9. Rotruck JT, Pope AL and Ganther HE. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179:588–90.
10. Kakkar P, Das B and Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Bio-chemistry and Biophysics.1984;21:130-2.
11. Sinha AK. Colorimetric assay of catalase. Analytical Biochem-istry.1972;47:389–94.
12. Lucchesi ME, Chemat F and Smadja J. Solvent free microwave extraction of essential oil from aromatic herbs: Comparison with conventional hydro-distillation. Journal of Chromatography A. 2004;1043:323-327.
13. Loziene K and Venskutonis PR. Influence of environmental and genetic factors on the stability of essential oil composition of Thy-muspulegioides. Biochemical systematics and ecology. 2005;33:517-525.
14. Ibrahim NM, Eweis EA, El-Beltagi HS and Abdel MobdyYE. Effect of lead acetate toxicity on experimental male albino rats. Asian Paci J TropBiomed. 2012;41-46.
15. Torres SJ and Nowson CA.Relationship between stress, eating behavior, and obesity. Nutrition. 2007;23(11):887-894.
16. Smith DM, Mielke HW and Heneghan JB. Subchronic lead feeding study in male rats. Arch Envion Contam Toxicol. 2008;3:518-528
17. Ming-Song T, Kai-Wei L, Chia-Huang C, Ling-ChuC, I-FangM, Yen-An T and Mei-Lien C. The critical fetal stage for maternal manganese exposure. Environmental Research. 2015;137:215–221.
18. Mostapha B, Djallal EHA, Miloud S, Wafaa A, NarimaneT and Khaled K.

Evaluation of anti-oxidative effects of Mentha spicata essential oils in rats at the period of gestation and lactation co-exposed to lead and manganese. South Asian Journal of Experimental Biology. 2019;8(5):167-171.
19. Toghyani M, Gheisari A, Ghalamkari GH and Mohammad RM. Growth performance serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (Nigella sativa) and peppermint (Menthapiperita). Livest Sci. 2010;129:173-178.
20. Zargari A. Medicinal plants. 2 edition; Tehran University press. Tehran. 2002.
21. Moreira EG, de Magalhaes RGJ, Barros SBM, Vassilieff VS and Vassillieff I. Antioxidant defense in rat brain regions after developmental lead exposure. Toxicology. 2001;169(2): 145-151.
22. Shukakidze A, LazrievI, and Mitagvariya N. Behavioral impairments in acute and chronic manganese poisoning in white rats. NeurosciBehav Physiol. 2003;33(3):263–7.
23. Annabi BA, Nehdi A, Hajjaji N, Gharbi N and El-Fazâa S. Antioxidant enzymes activities and bilirubin level in adult rat treated with lead. CR Biol. 2007;330(8):581-8.
24. Venkareddy L and Muralidhara M. Locomotor deficits and brain oxidative stress induced by lead (Pb) exposure are attenuated by eugenol supplements in prepubertal rats. Movement Disorders.2014;841-29.
25. Aschner M, Allen JW, Kimelberg HK, LoPachin RM and Streit WJ. Glial cells in neurotoxicity development. Annual Review of Pharmacology and Toxicology.1999;39(1):151-173
26. Latronico T, Branà MT, Merra E, Fasano A, Di Bari G, Casalino E and Liuzzi GM. Impact of manganese neurotoxicity on MMP-9 production and superoxide dismutase activity in rat primary astrocytes. Effect of resveratrol and therapeutical implications for the treatment of CNS diseases. Toxicological sciences. 2013;135(1):218-228.
27. Bhuvaneswari S, Murugesan S, Subha TS, Dhamotharan R and Shettu N. In vitro antioxidant activity of marine red algae. Chondrococcushornemanni and Spyridiafusiformis. Journal of Chemical and Pharmaceutical Research. 2013;5(3):82-85.
28. Martinez-Finley EJ, Gavin CE, Aschner M and Gunter TE.Manganese neurotoxicity and the role of reactive oxygen species. Free radical biology and medicine. 2013;62:65-75.
29. Hassan HA, Hafez HS and Isa AM. Neuro-protective effect of Mentha extract against oxidative stress in pre-treated gamma irradiated Swiss albino mice. Journal of Medicinal Plants Research. 2012;6(15):2995-3002.
30. Brahmi F, Madani K, Dahmoune F, Rahmani T, Bousbaa K, Oukmanou S and Chibane M. Optimization of solvent extraction of antioxidants (phenolic compounds) from Algerian mint (Mentha spicata L.). PhcogCommn. 2012;2(4):72–86.
31. Jajetia GC. Radioprotective potential of plants and herbs against the effects of ionizing radiation. J Clin Biochem Nutr. 2007;40(2):74–81.