Beneficial effect of *Mentha spicata* essential oil on lead and manganese induced nephrotoxicity and oxidative damage in rat kidney

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Abstract

Our work focused on the impact of chronic lead acetate and manganese chloride poisoning on renal function in Wistar rats. It also tested the efficacy of the essential oil extracted from (*Mentha spicata*) spearmint in reversing these effects by treating previously poisoned rats with a daily intraperitoneal injection of 0.1 ml HEM/kg over a 21-days period. Analysis of this essential oil through gas chromatography coupled with mass spectrometry revealed that the main components are: carvone (42.2%), menthone (20.89%), piperitenone (17.41%) and isomenthone (7.99%). Chronic oral co-exposure to lead and manganese during periods of gestation and lactation resulted in a significant reduction (P<0.01) in both body and kidney weight. Further examination of renal function revealed significant disturbances in the quantity of markers of renal function (creatinine, urea, uric acid) in the blood. Chronic co-exposure led to the observation of disruption to antioxidant enzyme (superoxide dismutase, glutathione peroxidase and catalase) activity in poisoned rats in comparison with the control rats; these results suggest renal failure. Indeed, histological study of the kidneys revealed pronounced damage manifesting as cell degeneration with the renal parenchyma affected by inflammation. In addition, the administration of the essential oil of *Mentha spicata* led to the observation of an increase in body weight, regulation of the various biochemical markers, and the regeneration of damaged renal tissue, all of which proves the high importance of spearmint in traditional medicine, and its therapeutic properties in combating renal problems.

Keywords: GC-MS; kidneys; lead; manganese; *Mentha spicata*; oxidative stress

Introduction

The heavy metals are a group of environmental chemicals which are both universal and non-biodegradable. Although the adverse effects resulting from exposure to them are well-known, their use and environmental concentration are increasing (Zhao *et al.*, 2014). Numerous studies have revealed the toxicity of individual metals for living organisms (Basile *et al.*, 2012). However, these metals do not only exist individually, but also combined in the environment (Smith *et al.*, 2012).

In fact, lead is a non-essential element for the human body, due to its dispersal in the ambient air, as well as in many foods. Its toxicity is closely tied to its accumulation in certain tissues, and its interference with bio-elements, whose role is essential for a number of physiological processes. It has numerous adverse effects, including neurological, behavioral, immunological, renal, hepatic, and in particular hematologic disorders (Annabi *et al.*, 2007).

As for manganese (Mn), an essential metal found in various biological tissues, it is required for the normal functioning of numerous physiological processes, including amino acids, lipids, and protein and carbohydrate metabolism (Erikson *et al.*, 2005). It also plays a vital role in the functioning of the immune system, cellular energy regulation, bone growth, and blood coagulation (Erikson *et al.*, 2003). However, in excessive quantities it may cause damage to the nervous system and enzyme activity (Aschner *et al.*, 2006).

In addition, the clinical importance of plant-based therapeutics (phytotherapy) has received considerable attention recently, as they are a rich source of medicines and produce a wide range of bioactive molecules (Small *et al.*, 2000). The significant nephroprotective activity of numerous medicinal plants and plant extracts has been reported in animal models (Lakshmi *et al.*, 2012). Among these plants *Mentha spicata L.*, commonly called "naana" in Algerian medical systems, has many culinary and medicinal uses in the Maghreb. It is popularly consumed in the form of tea and added to several preparations as a flavour enhancer; dry or fresh spearmint leaf is added especially during tea brewing. Biliary disorders, menstrual pain, stomach aches, constipation, gingivitis and waving are treated with decoction of spearmint leaves (Brahmi *et al.*, 2012).

All these properties, typical of mint species, have been attributed to the combination of essential oil, essentially based on monoterpenoids (Brada *et al.*, 2007), and polyphenolic derivatives (Dorman *et al.*, 2003).

This study examined the impact of chronic co-exposure to lead acetate and manganese chloride at the kidney level in Wistar rats during intrauterine life, lactation and after weaning, and then evaluated the effect of the essential oil of the plant *Mentha spicata* attenuated this nephrotoxicity in rats intoxicated by these two metals.

Materials and Methods

Extraction and determination of the chemical composition of the essential oil through GC/MS
Spearmint (*Mentha spicata*) leaves were harvested in Sidi Maamar, Saïda province, located on the high plateaus of western Algeria, before being identified by expert taxonomists (Prof. Hasnaoui and Dr. Sitayeb). The sample was preserved, while the voucher specimen, designated P-200886, was taken to the herbarium at the biology department of the faculty of sciences at the University of Saida, Algeria, for future reference. The analytical study of the essential oil of *Mentha spicata* was performed by gas chromatography (VARIAN CHROMPACK - CP 3900, injection of 0.1 µl of extract) at the quality analysis laboratory in Oran province. Helium (He) was used as the carrier gas at a flow of 1.2 ml/min. The column used was a Chrivasil-Dex CB fused silica CP-type capillary column with a length of 30 m and an internal diameter of 0.25 mm. Thickness in the stationary phase was 0.25 µm; the initial injection temperature was set to 70 °C for 2.5 minutes, then increased by increments of 5 °C/min up to 280 °C; a mass spectrometer (Saturn 2200) was used as the sensor for this analysis, with a temperature of 280 °C. The apparatus was operated by a computer guided by software suited to this type of analysis, and an NIST data bank which enabled the identification of the compounds.

Distribution of lots
The experiments were carried out on Wistar rats, weighing from 200 to 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, at a temperature of 21 °C ± 1 °C with artificial lighting 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 D_0 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 $(Pb(C_2H_3O_2)_2)$ at 0.2% and manganese chloride tetrahydrate $(MnCl_24H_2O)$ at 4.79 mg/ml in bidistilled water orally from the first day of gestation until weaning (n = 07 male rats) (Kahloula *et al.*, 2009; Molina *et al.*, 2011; Brahmi *et al.*, 2018).
- Group control: consisting of animals which receives the distilled water. Tested offspring are subject to the same conditions as their mother.
- Group T-HEM: 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-HEM: 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) (Halder *et al.* 2011; Brahmi *et al.*, 2019).
- The number of suffering animals were minimized in accordance with the guidelines of the European Council Directive (86/609/EEC).
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Body and kidney weight

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

Biochemical tests

Lead and manganese analysis in tissues of kidney

Depositing 1g fresh weight of each sample with 1 ml of nitric acid (HNO_3) at 65% purity, we bring the temperature to 95 °C for one hour, after cooling; we supplement the content to 4ml of double distilled water. The lead and manganese concentrations were determined in the organs by atomic absorption spectrophotometry (SHIMA DZU AA6200) and the values were expressed in $\mu g\,g^{-1}$

Determination of kidney parameters

Serum concentrations of urea, uric acid and creatinine (Murray and Kaplan, 1984) were determined colorimetrically as measures of kidney functions.

Measuring the activity of antioxidant enzymes

The kidney 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 1000 x g for 15 min at 4 °C. The resulting supernatant was also centrifuged at 1000 x g for 15 min at 4 °C. The pellet consisted of the mitochondrial fraction, and the supernatant was re-centrifuged at 10.000 x g for 30 minutes. The two resultant pellets were made soluble in a buffer solution containing 0.32 M sucrose, 0.5 mM EDTA, 10 mM Tris-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 10.000 x g for 15 min 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) (Michaux, 1971). The superoxide dismutase (SOD) (EC 1.15.1.1) was analyzed in the supernatant using the technique of Kakkar (1984); 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) and Rotruck, (1973), respectively.

Histological study

Samples of Kidney spleen were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Paraffin sections (5 μm thick) were prepared, routinely stained with hematoxylin and eosin (H&E) dyes (Suvarna *et al.*, 2013), and then examined microscopically

Statistical analysis

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).

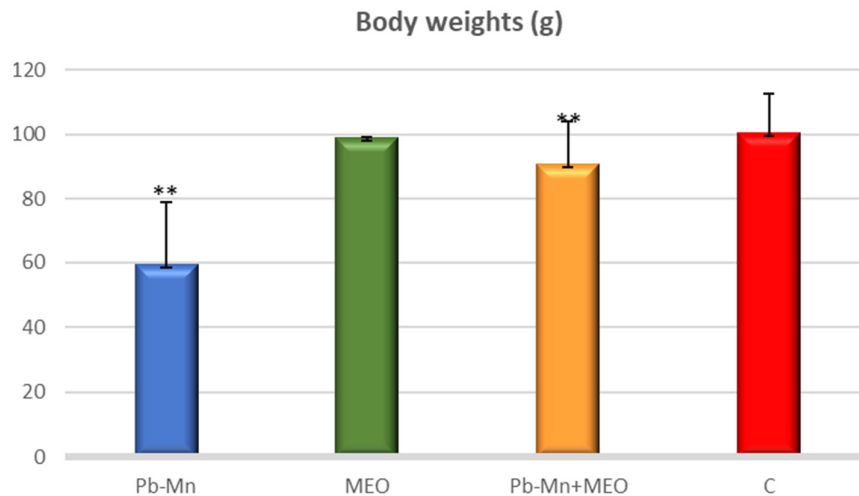## Results

The yield of essential oil

The hydro-distillation of the plant matter *Mentha spicata* allowed to obtain a yield of 0.83%. The analysis of the essential oil of *Mentha spicata* by gas chromatography identified 12 major compounds listed in Table 1 by order of elution. Twelve components representing the sum of the percentages of the components obtained were identified (98.51%), of which 96.71% are monoterpene ketones, 1.75% are monoterpene hydrocarbons, 0.05% are oxides. The major components of this oil are: carvone (42.2%), menthone (20.89%), piperitenone (17.41%) and isomenthone (7.99%) (Table 1).

Table 1. Concentration in % and retention time of the different compounds obtained by gas chromatographic analysis of the essential oil of *M. spicata*

| Compounds	Retention time (min)	Concentration (%)
α-pinene	11.200	0.40
Sabinene	12.080	0.56
β-pinene	12.440	0.70
Octane-3-one	12.820	1.15
Paracymen	13.600	0.09
1.8 cineole	14.110	0.05
Menthone	18.213	20.89
Isomenthone	18.710	7.99
Pulegone	21.460	0.05
Carvone	21.480	42.2
Piperiton	22.010	7.02
Piperitenone	23.150	17.41
Total of identifiers		98,51

Effects on the body and the kidney 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 (Figure 1).

Figure 1. Effect of *M. spicata* essential oil on the body weight in rats intoxicated (Pb-Mn)
Data are mean ±S.E.M. ** P<0.01, (Pb-Mn vs. C).

The results found in the poisoned animals also reveal a significant decrease (p < 0.01) in kidney weight compared to the control group (Figure 2). 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).
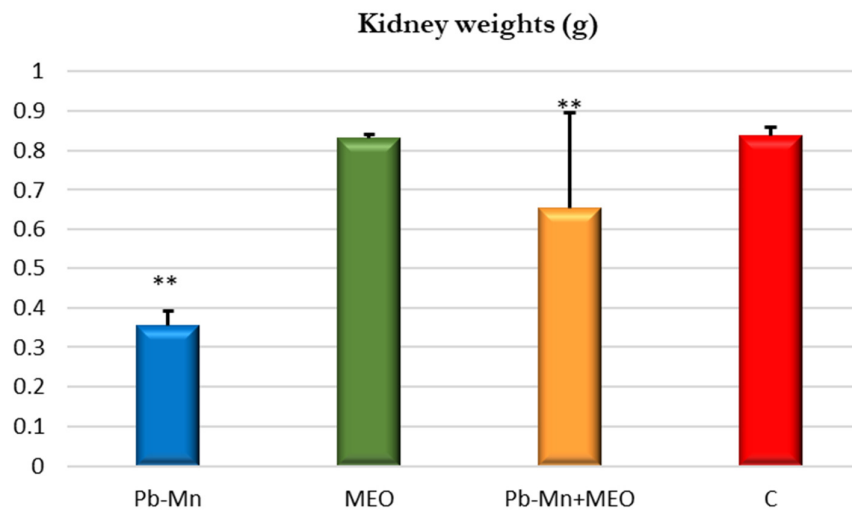
Figure 2. Effect of *M. spicata* essential oil on the kidney weight in rats intoxicated (Pb-Mn)
Data are mean ±S.E.M. ** P<0.01, (Pb-Mn vs. C).

The results show that the concentration of lead and manganese in the kidney was significantly higher (p < 0.01) in the poisoned rats compared to the control rats. The administration of the essential oil led to the recording of a significant reduction (p < 0.01) in the levels of lead and manganese in the kidneys (Table 2).

Biochemical analysis of the renal biomarkers at the end of the experiment showed a significant increase in creatinine, urea and uric acid in the rats exposed to Pb-Mn compared to the control rats. This shows that these two metals lead to renal dysfunction (Table 2). After 3 weeks of MEO treatment, we observed a clear correction of the concentration of creatinine, urea and uric acid in the plasma of the previously poisoned rats.

Table 2. Effect of the essential oil of the *M. spicata* plant on the various biochemical parameters in rats intoxicated by Pb-Mn compared to control rats

	Pb-Mn	MEO	Pb-Mn+MEO	C
Pb (µg/g)	40.41±0.15*	0.55±0.17	21.13±0.10*	0.51±0.08
Mn (µg/g)	138± 0.02**	99±0.04	111.2 ±0.1**	93±0.10
Urea (mg/dl)	4.40±0.20***	2.89±0.20	3.99±0.29***	2.72±0.18
Uric acid (mg/dl)	6.11± 0.45**	3.017± 0.26	4.14± 0.25**	3.50±0.56
Creatinine (mg/dl)	10.84±0.25**	7.55± 0.22	8.33± 0.05**	7.40± 0.38

Data are mean ± SEM (***: $p<0.001$, **: $p<0.01$, *: $p<0.05$)

Renal antioxidant enzyme activity

The renal antioxidant results show a significant decrease in SOD, CAT and GPx in the kidneys 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 3).

Table 3. Effect of *M. spicata* essential oil in the activity of anti-oxidant enzymes in kidney of rats intoxicated

| Groupes	Pb-Mn	MEO	Pb-Mn+MEO	C
SOD (U/g)	0.51±0.059*	0.95±0.09	0.79 ±0.019*	1.09±0.02
CAT (U/g)	0.36 ±0.34*	0.53 ± 0.07	0.50 ±0.02*	0.65 ± 0.05
GPx (U/g)	0.73± 0.03**	0.22±0.005	0.83± 0.05**	1.23± 0.33

Data are mean ± SEM (**: $p<0.01$, *: $p<0.05$).

Effect of Pb-Mn on the structural architecture of the kidney

The histological study of renal tissue in rats suffering from Pb-Mn poisoning (Figure 3B) shows considerable tubular degeneration with renal failure. The organization of the tissue presented a renal parenchyma affected by inflammation caused by active congestion and an edema. There was also evidence of partial necrosis of the convoluted tubules (presence of homogeneous cytoplasm cells lacking nuclei).

In the same context, the animals exposed to Pb-Mn and treated with MEO presented regeneration of the tissue with a renal parenchyma showing signs of a minor inflammatory reaction caused by minor inflammatory lymphocytic infiltrate (Figure 3C).

Furthermore, observation through an optical microscope of the renal tissue of the control rats and the rats treated with MEO showed a normal structure in the renal tubules and glomeruli (Figure 3 A-D).
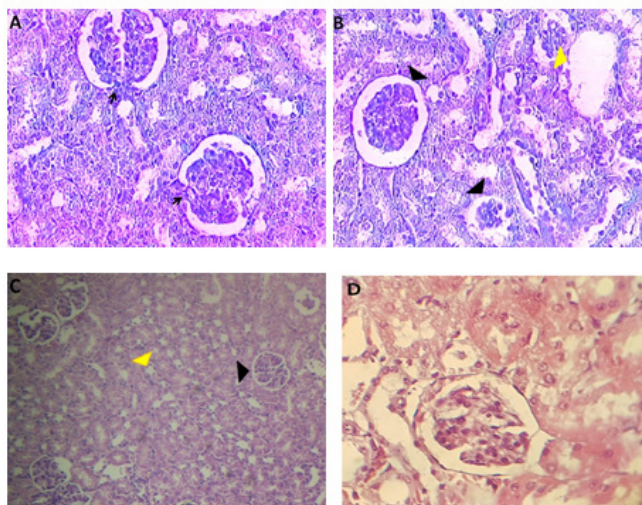
Figure 3. Section of renal rat tissue with hematoxylin and eosin (G:x20); **(A)** normal architecture and normal Bowman capsule (➡) in the control group (T) (Animals received distilled water) and the group MEO **(D)**,(Animals received essential oil); **(B)** group (Mn-Pb), (Intoxicated animals) Showing degeneration of Bowman capsules (▲), with large interstitial spaces and complete absence of bypassed tubes (△) ; **(C)** the group exposed to (Mn-Pb) and treated with essential oil, Showing the regeneration of the majority of Bowman's capsules (▼) and interstitial cells, with convoluted tubes (△)

Discussion

The essential oil of *Mentha spicata* (MEO) has been obtained through hydro-distillation with a yield of 0.83%, a figure that contradicts the work of (Priscilla *et al.*, 2010), which reported a value of (0.23±0.4%) and that of (Lucchesi *et al.*, 2004) (0.60±0.06%). This difference in yield can be attributed to multiple factors, the most significant being the origin, species, harvesting time, length of drying period and technique used to extract the essential oils (Karousou *et al.*, 2005). Furthermore, Marotti *et al.* (1994) have shown that weak yields can be associated with a series of factors such as the genotype, the growth stages of the plant, and the environmental conditions of semi-arid regions.

Nevertheless, the GC-MS of this herb first and foremost includes an essential oil with various chemical components of diverse natures, such as Carvone (42.2%), Menthone (20.89%), Piperitenone (17.41%) and Isomenthone (7.99%). These results corroborate those of (de Sousa Barros *et al.*, 2015), which show that this oil consists principally of carvone.

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 (Torres and Nowson, 2007; Ibrahim *et al.*, 2012). 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 kidney 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 (Smith *et al.*, 2008; Ming *et al.*, 2015).

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 (Vinay Dwivedi *et al.,* 2011). Our

observations are also in keeping with those of (Toghyani *et al.,* 2010*)*, 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. (2002); 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.

The results recorded for poisoned rats showed an accumulation of Pb and Mn in the kidneys. Han *et al.* (1996) has suggested that this factor is closely linked to macromolecules in the intracellular compartment, as lead-binding proteins have been isolated from the kidney, liver, blood and brain, explaining the high concentration of Pb in the kidney tissue found in this study.

In our study, we have observed a significant increase in the levels of urea and creatinine in the blood of animals with Pb-Mn poisoning. These results are in keeping with the work of (Thylambal and Saroja, 2004; Abdel Moneim *et al.*, 2011), who have shown that the renal cells are no longer capable of controlling the urinary excretion process, as the kidneys are among the organs most sensitive to lead. In addition, according to the study of Saka *et al.* (2011), the increase of serum urea and creatinine in rats exposed to Pb is due to the degradation of hepatic protein

Compounds, whereby the proteins may be broken down into amino acids, and then again into urea and creatinine. In this way, when exposed to serum aminotransferase, these amino acids may be converted into carboxylic compounds such as pyruvic acid. According to (Michaux *et al.*, 1971), this is the result of a reduction in the rate of glomerular filtration.

Even so, Sánchez-González *et al.* (2015) have found that Mn also plays a role in chronic renal failure, which in turn causes an increase in urea and creatinine levels, which may be due to the adverse effect of manganese on calcium ions (Ca^{+2}) during the glomerular filtration processes. The results obtained are also corroborated by those of Madlala *et al.* (2012); Sánchez-González *et al.* (2015) who found that Mn plays a role in chronic renal failure, which leads to an increase in urea and creatinine levels. This hypercreatininemia may be a result of the accumulation of manganese in the various cellular compartments of the kidney.

Endogenous antioxidant enzymes are responsible for the neutralization of free radicals, as well as preventing their damaging effect on tissue. Oxidative stress is a result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, which in turn leads to a series of events deregulating cell functions. Antioxidant enzymes such as SOD, CAT and GPx form a united defensive front against ROS (Bandyopadhyay *et al.*, 1999). Our results show that lead acetate and manganese caused a significant reduction in the concentration of SOD, CAT and GPx in the kidneys of rats suffering from Pb and Mn poisoning in comparison with the control rats.

It has been demonstrated that the accumulation of lead in the various cellular compartments of the liver leads to oxidative damage through an increase in lipid peroxidation and protein oxidation, a deleterious process caused only by free radicals (Villeda-Hernandez *et al.*, 2001; Kong *et al.*, 2004).

The activity of superoxide dismutase (SOD) is significantly reduced in the renal tissue of the poisoned rats compared to the controls group, which could be due to the mass production of superoxide anions and a deficiency of copper as an enzymatic cofactor (Matés *et al.*, 2000). SOD plays a very important role in the antioxidant system through the disproportionation of the superoxide anion to form H_2O_2 and O_2 (Mylorie *et al.*, 2001).

The reduction in catalase in the kidney tissue of poisoned rats compared to the control rats could be due to the effect of lead on the active components of the cellular defense system through a change in the activity of said enzyme (Alghazall *et al.*, 2008).

Numerous works suggest that exposure to Mn leads to various cellular changes, due to an increase in oxidative stress and the failure of the metabolic energy and antioxidant systems (Dobson *et al.*, 2004).

Recent studies have revealed the link between oxidative stress and mitochondrial dysfunction resulting from exposure to this metal (Mn) (Milatovic *et al.*, 2007). Furthermore, one particular hypothesis has been posited suggesting that Mn poisoning is associated with mitochondrial dysfunction, which builds up

preferentially in the mitochondrial matrix (Liccione et Maines. 1988), reflecting its high affinity with calcium (Gavin *et al.*, 1999).

Oxidative stress caused by high concentrations of Mn leads to the induction of pores to open and the mitochondrial permeability of said pores, dependent on Ca^{+2}, which leads to an increase in the solubility of protons, ions and solutes, the potential loss of the internal mitochondrial membrane, a decline in the effects of oxidative phosphorylation and ATP synthesis, and mitochondrial swelling (Yin *et al.*, 2008).

Multiple observations have demonstrated that intracellular GSH levels are affected by Mn poisoning, and that deficiency in mitochondrial function is a key mechanism in Mn poisoning (Dukhande *et al.*, 2006). For example, Mn^{+2} can replace Fe^{+2} in the cytochromes of the cellular respiration chain (with a similar structure to hemoglobin) (Missy *et al.*, 2000), leading to the incomplete reduction of O_2 and the formation of free radicals and oxygen compounds such as O_2 and H_2O_2.

It has been demonstrated that Mn directly inhibits enzymes of the mitochondrial ETC (Electron Transport Chain) (Singh *et al.*, 1979). It could be predicted that this inhibition may also improve the production rate of radicals. An increase in the production of radicals would be further exacerbated by the decrease in GPx and the activities of SOD (Liccione et Maines. 1988).

Effect of Pb-Mn on the structural architecture of the kidney

In this study, the results of the microscopic examination of histological sections taken from the kidneys of rats suffering from Pb-Mn poisoning revealed that the renal parenchyma was affected by inflammation caused by active congestion, an edema, and the partial necrosis of the convoluted tubules. The control rats, on the other hand, presented a normal renal parenchyma, including the renal glomeruli and convoluted tubes, all within interstitial tissue showing no irregularities. These results are in keeping the work undertaken by (Abdel Moneim *et al.* 2011; Zhang *et al.* 2013) who showed that Pb causes histological changes in the kidneys, which develop and are notably characterized by a decrease in glomeruli in the kidney, an inflammatory reaction, cytoplasmic vacuolization, and the dilatation of certain renal tubules. These tubular deformations caused by poisoning could be the result of a hydraulic change in the renal tissue, and suggest that lead poisoning leads to the partial failure of ion pumps in the tubular cells, which in turn causes tubular swelling, leading to necrosis and tubular vacuolization (Karmakar *et al.*, 1986; Wei *et al.*, 2001)

Exposure to manganese chloride causes changes to the renal architecture (inflammation, tubular necrosis). This damage invokes the hypothesis of a state of oxidative stress caused by manganese within the cells (Guilherme *et al.*, 2015). Knowing that manganese can replace different oligo-elements of the same valency within metalloproteins (enzymes), which require the presence of said oligo-elements in their activities and subsequently cause disruption in the various metabolisms.

However, mint is the most popular medicinal plant in the world, and its essential oil is a key part of aromatherapy (Schneider *et al.*, 2000). Spearmint, or *Mentha spicata* to use its scientific term, is very widely distributed in Algeria, especially in the province of Saïda.

For this reason, the aim of our work is to assess the therapeutic effect of the essential oil of the *Mentha spicata* plant in cases of lead and manganese poisoning in Wistar rats over the course of the period of development (gestation and lactation).

The administration of MEO to the poisoned rats has shown that treatment with the oil reduced considerably the concentration of lead and manganese in the kidneys. This effect may be due to the presence of chelate compounds, as well as the redox properties of the polyphenols, which allow them to act as antioxidants and reduce the accumulation of the aforementioned elements (Kaisoon *et al.*, 2011).

Furthermore, following the treatment of rats suffering from Pb-Mn poisoning with MEO, we noticed a reduction in the levels of urea and creatinine; our results are in keeping with the work of Bellassoued *et al.* (2018) which concluded that the essential oil of the spearmint plant provides a protective effect against hepato-nephrotoxicity.

In the same context, the histological results for the kidneys of rats suffering from Pb-Mn poisoning and treated with MEO have allowed us to observe a clear improvement in comparison with the untreated rats. The efficacy of MEO in the kidney was perhaps due to the presence of chelate compounds (Ullah *et al.*, 2014). These biologically active compounds can chelate the Pb-Mn and increase its excretion from the organism, thus reducing the accumulation of Pb and Mn in the renal tissue.

Ullah *et al.* (2014) has also shown that mint extract (*Mentha piperita)* reduces tubular necroses in the kidneys induced by gentamicin, and provides protection against renal failure by acting as an antioxidant.

Additionally, the intraperitoneal administration of MEO at a dose of 0.1 ml/kg has allowed us to observe a significant increase in SOD and CAT content, and in the activity of GPx in rats exposed to Pb-Mn. Our results are in keeping with Singh *et al.* (2014); Azab *et al.* (2017); Bellassoued *et al.* (2018) which indicate that the force-feeding of hydroalcoholic extract of mint (*M. arvensis, M. piperita*) led to a significant increase in SOD content and GPx activity in rats previously poisoned with CCl_4.

Pharmacological studies have shown that the essential oils derived from various plant materials possess anti-inflammatory properties (Kim *et al.*, 2008; Martins *et al.*, 2008). Knowing that terpenes have excellent anti-inflammatory properties (Cowan *et al.*, 1999), the anti-inflammatory properties of the essential oil derived from the leaves of *M. piperita* can in part be explained by the presence of said terpenes. Furthermore, menthol and isomenthone, found in relatively high concentrations in the essential oil of *M. piperita*, have been reported to provide anti-inflammatory activities (Cowan *et al.*, 1999).

Bellassoued *et al.* (2018) have shown that pretreatment with the essential oil of *M. piperita* at 40 mg kg^{-1} can also lead to a clear improvement in histopathological kidney damage induced by the administration of CCl_4. Other works have revealed that *M. arvensis* provides a nephroprotective effect against the nephrotoxicity of cisplatin in Sprague-Dawley rats, which could be attributable to the presence of flavonoids and related compounds (Singh *et al.*, 2014).

Conclusions

Exposing Wistar rats to Pb-Mn during gestation and lactation revealed nephrotoxic effects which resulted in a significant change to the antiradical system, represented by various enzymes. Treatment with MEO for previously poisoned rats showed significant improvement in the histopathological image of the kidney, as well as general renal function.

Authors' Contributions

BM and ADEH designed and performed the experiments and also wrote the manuscript. ZK performed the biochemical and statistical analysis. HH carried out the histological study. SM and KK reviewed the manuscript. All authors read and approved the final version.

Ethical approval (for researches involving animals or humans)

The number of suffering animals were minimized in accordance with the guidelines of the European Council Directive (86/609/EEC).

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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