Neuroprotective effect of *Foeniculum vulgare* essential oil in developing Wistar rats exposed to manganese chloride

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**Abstract**

The present study was carried out in order to evaluate, on the one hand, the modifications induced by manganese chloride according to a neurobehavioral, biochemical and histological approach in developing Wistar rats and, on the other hand, to test the effectiveness of *Foeniculum vulgare* (fennel) essential oil (FEO) in restoring or not the harmful effects of manganese chloride (MnCl2). The characterization of this essential oil by gas chromatography showed that the major component is E-anethole (34.7907%). The administration of FEO corrected the depressive state, anxiety and locomotor hypoactivity respectively observed in rats exposed to MnCl2, thus, the FEO restored the activity of the various antioxidant enzymes with a clear improvement of the cerebral tissue architecture in intoxicated rats treated with FEO. In conclusion, the FEO has a beneficial effect on the nervous system of rats intoxicated with MnCl2 which justifies the importance of this oil in traditional medicine.

**Keywords:** Manganese; *Foeniculum vulgare*; GC-Ms; neurobehavioral tests; brain; rat.

**INTRODUCTION**

Manganese (Mn) is one of the most abundant metals on the earth’s surface, covering about 0.1% of the earth’s crust. Mn is found in pure form but also associated with more than 100 minerals (ATSDR, 2000). Humans can be exposed to Mn in four ways: through water, food, air or contact with industrial products (Daoust, 2012). The main manifestations of its chronic toxicity affect the central nervous and are manifested by neurological disorders of the Parkinsonian type (Iarc, 2006).

Therefore, the clinical importance of herbal therapeutics (phytotherapy) has received considerable attention lately as they are a rich source of medicines because they produce a quantity of bioactive molecules (Small et al., 2000). Produced as secondary metabolites by plants, essential oils are used in several fields; they have found their place in aromatherapy, pharmacy, cosmetics and food preservation (Teixeira et al., 2013). Fennel (*Foeniculum vulgare*) has been used for decades for its culinary and medicinal virtues (Hendawy et al., 2010). In addition, other properties have been discovered, such as antispasmodic, diuretic (Shabat et al., 2011), hepatoprotective (Ozbek et al., 2003), anti-inflammatory, analgesic, and antioxidant (Badgujar et al., 2014). The main types of anti-microbial (Majdoub et al., 2017), anti-microbial, anti-fungal (Roby et al., 2013), anti-diabetic (Saleem et al., 2017), anti-neurological disorders (Cioanca et al., 2016) and anti-cancer (Anand et al., 2008). In the light of these data, our study is aimed at investigating the effect of the essential
oil of the *Foeniculum vulgare* plant on the neurotoxicity induced by manganese chloride in Wistar rats.

**MATERIALS AND METHODS**

**Extraction and determination of the chemical composition of the essential oil by GC/MS**

The fennel grains (*Foeniculum vulgare*) were harvested from Saïda in the highlands of western Algeria, and then identified by taxonomic experts (Sitayeb, 2018). The sample was preserved, and the specimen voucher, coded P-201976, was deposited in the herbarium of the Biology Department of the Faculty of Sciences of the University of Saïda, Algeria, for future reference. The essential oil of *Foeniculum vulgare* was extracted by hydrodistillation (Rather et al., 2016). The analytical study of the essential oil of *Foeniculum vulgare* was carried out by gas chromatography type VARIAN CHROMPACK - CP 3900 by injection of 0.1 µl of extract. The carrier gas used is helium He with a flow rate of 1.2 ml/min. The column used is a CP type capillary column a Chirasil-Dex CB Fused silica, 30 m long and 0.25 mm inner diameter. The thickness of the stationary is 0.25 µm; the temperature of the initial injection column was programmed to be 70 °C for 2.50 min and then rises in steps of 5 °C/min at 280 °C; the detector used for this analysis was a mass spectrometry type detector (Saturn 2200) with a temperature of 280 °C. The apparatus is controlled by a menu-driven computer with software suitable for this type of analysis and a NIST database that allows the identification of compounds.

**Experimental Animals**

The experiments are carried out on albino rats of the Wistar strain, weighing between 200 and 350 grams, housed in the animal house of the Department of Biology (University of Saïda). The rats are grouped in groups of 3 in Makrolon cages (LxWxH = 40×25×18 cm) with 2 females and 1 male, placed in a ventilated room, at a temperature of 21°C. The animals have *ad libitum* access to food (rodent croquet) and a bottle filled with tap water. Artificial lighting establishes a day/night cycle (daytime between 7 AM and 7 PM). Experiments were carried out between 9 AM and 6 PM.

**Preparation of injectable solution**

According to the work of Halder et al. (2011), injected (FEO) must be diluted in doubly distilled water with a few drops of Tween 80 to obtain a solution at a concentration of 0.1 ml/kg.

**Distribution of groups**

At D0 of gestation the females were divided into two groups:  
*Group Mn*: constituted by animals exposed to manganese, contained 9 rats, 3 males and 6 females (weighing between 200 and 350 grams), receiving orally manganese chloride tetrahydrate (MnCl$_2$4H$_2$O) solubilized in double distilled water at a rate of 4.79 mg/ml (Adli et al., 2017).  
*Group Control*: the control group contained 9 rats, 3 males and 6 females (weighing between 200 and 350 grams) received only the bi-distilled water. Tested descendants were subjected to the same conditions as their mother. 24 hours after weaning the animals were divided into quarter groups:  
*Group Mn*: rats exposed to manganese (Mn: N = 7; Wb: 70g).  
*Group Mn-FEO*: rats exposed to manganese and treated with *Foeniculum vulgare* essential oil (FEO) (0.1 ml/kg) (Mn-FEO, N = 7; Wb: 70g) at the dose of an intraperitoneal injection per day for 21 days, 30 minutes before the behavioral test.  
*Group FEO*: treated with the essential oil of *Foeniculum vulgare* (FEO) alone (0.1 ml/kg) (FEO: N = 7; Wb: 70g).  
*Group Control*: rats receive bi-distilled water (N = 7; Wb: 70g).  
The number of suffering animals was minimized in accordance with the guidelines of the European Council Directive (86/609/EEC).

**Behavioural tests**

*Forced Swimming Test*

The forced swimming test was originally proposed by Porsolt *et al.* (1977), as a test for the selection of molecules with antidepressant activity. Rats were placed for 15 minutes in the room where the test takes place. The animals were subjected to a 6-minute forced swimming test. They were placed inside a cylinder of 20.7 cm in diameter and 39 cm in height in water at 22°C. The length of time the animal actively swims or only floats so that its head is kept above water was measured (Figure 1). After being tossed in the water, the animal becomes almost immobile, moving its legs from time to time to stay afloat or regain its balance. This immobility is interpreted as a reflection of "behavioral despair", which occurs when the animal realizes that it will not be able to escape. In this interpretation, immobility is seen as depressive behavior.
Elevated plus maze

The Elevated plus maze is a measure of an animal's degree of anxiety based on its spontaneous aversion to emptiness. The experiment exploits the conflict, in rodents, between the fear of open spaces and the desire to explore a new environment. Closed arms represent safety, while open arms offer exploratory value. An anxious animal will naturally tend to prefer closed, dark spaces to open, bright spaces. Based on this principle, behavioral anxiety is measured by the degree of avoidance of the open spaces of the labyrinth. This model was originally described by Pellow et al. (1985) in rats. The device consists of four black-painted wooden arms (L = 50 cm and W = 10 cm) which communicates through a central area (10 x 10 cm). Two opposite arms have side walls 30 cm high (closed arms) while the other two have no side walls (open arms). The whole device is placed 53 cm above the floor and illuminated by a halogen lamp. The animal is placed in the central area, facing a closed arm. The number of entries as well as the time spent in each compartment is measured during a period of 5 minutes.

Open field test

This test is performed to evaluate the locomotor activity of rats. The arena used was an open rectangular box (90 × 70 × 60 cm), with a black background, and white lines on the bottom to delimit the tiles (10 × 10 cm). Each rat was initially placed in one of the four corners of the open field, with its head facing one corner. Its behaviour was observed for six minutes; between each test, the cage is cleaned with 70% ethanol. Six parameters were measured by the experimenter: the latency time (expressed in seconds), the number of visits to the nine central tiles, the total number of tiles passed through, the total number of grooming sessions, the total number of straightenings and the total number of defecations. This test analyzes the exploratory behavior of the rat in an enclosed space. It is used primarily to measure motor function, but also to assess anxiety levels. An anxious animal avoids the center of the field, which is open, and stays at the periphery (Adli et al., 2014).

Body weight evaluation

The monitoring of the young rats required a daily weighing of body weight throughout the experimental period from their weaning until the end of the experiment. The young rats were then sacrificed and the weight of the brain organs was recorded. This organ was used for the histological study.

Biochemical tests

Measurement of Mn brain level

After weaning and treatment with Foeniculum vulgare essential oil, the animals were beheaded and whole blood samples (100 μl) was collected in a 5 ml hemolysis tube containing a volume of 100 μl of 0.1% newt. After vortex agitation for 30 seconds, deproteination was achieved by the addition of 600 μl of HNO₃ (1M). This was followed by a second vortex agitation of the tube contents for 10 minutes at room temperature. The next step was the centrifugation of the total tube container at 3000 rpm for 10 minutes. At the end the blood lead level was determined by a type atomic absorption spectrophotometer (SHIMA DZU AA6200).

Determination of alkaline phosphatase (PAL)

The brains of the rats were placed in a 4 ml Potter-Elvehjem homogenizer. Homogenization was performed in ten volumes of 0.32 M sucrose ice solution. The entire homogenate and the rinsing liquid were centrifuged at 1000 g for ten minutes at 4 °C. The supernatant thus represents a crude synaptosomal fraction which was removed and kept in ice for enzyme determination (Adli et al., 2017). The concentration of released p-nitrophenyl is proportional to the activity of PAL and is measured photometrically at a wavelength of 405 nm.

Measurement of the activity of antioxidant enzymes

The brains of the recovered rats were homogenized in a buffer solution containing 0.5 mM EDTA, 10 mM Tris-HCl (pH: 7.4) 0.32 M sucrose, at 4 °C (1 mg tissue per 4 ml buffer solution) using a glass/glass homogenizer. The homogenate was centrifuged at 1000 x G for 15 minutes at 4 °C. The resulting supernatant was then centrifuged at 10,000 x G for 15 minutes at 4 °C. The pellet formed the mitochondrial fraction, and the supernatant was re-centrifuged at 10,000 x G/30 minutes. The two pellets thus obtained are solubilized in a buffer solution containing 0.5 mM EDTA, 10 mM Tris-HCl (pH 7.4), 0.32 M sucrose and 0.02% digitonin (pH 7.4); the second pellet was centrifuged at 10,000 x G for 15 minutes at 4 °C. In the end the pellet obtained, which constitutes the total fraction of mitochondria, was solubilized in a sucrose-containing solution (0.32 M at pH = 7.4) (Rottruck et al., 1973). Superoxide dismutase (SOD) [EC 1.15.1.1] was analyzed on the supernatant using the technique of Kakkar et al. (1984). The activities and levels of antioxidant enzymes in the brain, such as glutathione peroxidase (GPx) and catalase (CAT) were analyzed by the method of Rottruck et al. (1973).
Histological study

After sacrifice, brain samples were taken from all groups of rats and fixed in a buffered formalin solution (10%), dehydrated in ascending grades of ethanol (70-100%), clarified in xylene and cast (kerosene), blocked, cut to a thickness of 5 µm, after routine staining with haematoxylin and eosin (H&E) dyes (Bancroft, 1975), and then examined microscopically with magnification (×40).

Expression and statistical analysis of results

The results were expressed as the mean (M) of the individual values, assigned the standard error to the mean (S.E.M.). The comparison of several means was carried out by an analysis of variance (ANOVA) with the intoxication factor (Mn, T) and/or the treatment factor (HE, Mn) followed possibly by the Post-Hoc Student-Newman-Keuls test. A probability *P < 0.05 indicates a significant difference. **P < 0.01 indicates a highly significant difference; ***P <0.001 indicates a highly significant difference compared to controls. Statistical analyses were performed using Sigma Stat software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSIONS

The yield of essential oil

After the hydro-distillation of the fennel plant material, the yield was calculated by the ratio between the weight of oil extracted and the weight of plant material used, and was expressed as a percentage. Each percentage (%) is equal to 1.22%. This is in accordance with the research work of El-Sayed et al. (2015), which reported yields of about 1.52%.

Principal essential oil compounds detected GC/MS

The analysis of fennel essential oil by gas chromatography allowed the identification of the major components listed in (Table 1) in order of elution. 14 components representing the sum of the percentages of the components obtained were identified of which 20.2717% are mono-terpene hydrocarbons, 14.9548% are oxygenated monoterpenes, 36.0574% are arylpropynes and 28.211% are unknown substances. The major component of this oil is: E-anethole (34.7907%). Our results agree with the work of Rather et al. (2016), which show that the composition of the essential oil of *Foeniculum vulgare* presents a great chemo-diversity depending on the extraction method and geographical origin. As a result, the accumulation of these volatile compounds within the plant is variable, appearing practically in one of its parts, namely, roots, stem, shoots, flowers and fruits (Díaz-Maroto et al., 2006). Another study reported that the essential oil content and composition varies during the different stages of ripening of *Foeniculum vulgare*. The essential oil content that was reported decreased with the maturity of the fruit as the E-anethole content is the main component (Telci et al., 2009). In addition, a study conducted by Tognolini et al. (2007) indicates that phenylpropene estragole and trans-anethole, which are the major constituents of the oleoresin in the aerial parts of *F. vulgare* varies during the development of the plant, these two compounds being maximal in the flowers and mericarp development. The pharmacological effects of *F. vulgare* fruits are generally attributed to their essential oil.

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

<table>
<thead>
<tr>
<th>Determination</th>
<th>Retention Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>8.95 min</td>
<td>2.4549 %</td>
</tr>
<tr>
<td>Myrcene</td>
<td>10.54 min</td>
<td>1.4335 %</td>
</tr>
<tr>
<td>Unknown substance 1</td>
<td>11.13 min</td>
<td>12.792 %</td>
</tr>
<tr>
<td>α-phellandrene</td>
<td>11.35 min</td>
<td>1.3325 %</td>
</tr>
<tr>
<td>p-cymene</td>
<td>11.75 min</td>
<td>5.1070 %</td>
</tr>
<tr>
<td>Limonene</td>
<td>11.92 min</td>
<td>9.5032 %</td>
</tr>
<tr>
<td>Y-terpinene</td>
<td>12.86 min</td>
<td>0.4406 %</td>
</tr>
<tr>
<td>Fenchone</td>
<td>13.94 min</td>
<td>14.0556 %</td>
</tr>
<tr>
<td>Unknown substance 2</td>
<td>17.35 min</td>
<td>14.2330 %</td>
</tr>
<tr>
<td>Estragole</td>
<td>18.93 min</td>
<td>1.2667 %</td>
</tr>
<tr>
<td>Verbenone</td>
<td>19.13 min</td>
<td>0.8992 %</td>
</tr>
<tr>
<td>E-anethole</td>
<td>20.11 min</td>
<td>34.7907 %</td>
</tr>
<tr>
<td>Unknown substance 3</td>
<td>20.43 min</td>
<td>0.3482 %</td>
</tr>
<tr>
<td>Unknown substance 4</td>
<td>21.41 min</td>
<td>0.8378 %</td>
</tr>
</tbody>
</table>
Effect of Mn and FEO on body weight and brain weight

Statistical analyses showed a significant difference (P <0.001) between the weights of young Mn intoxicated rats compared to young control rats, which was due to a decrease in food intake throughout the experiment. In fact, it has been shown that manganese intoxication induces considerable changes in food intake. We also observed a reduction in brain weight in rats exposed to Mn compared to control rats (Table 2). This is in favor of a disturbance in their functioning. Our results are in agreement with the work undertaken by Adli et al. (2014) showing that manganese has an inhibitory effect at the center of satiety and hunger regulation which is the hypothalamus.

In fact, the administration of *Foeniculum vulgare* (fennel) intraperitoneally to rats exposed to Mn showed an increase in body gain compared to Mn poisoned rats. This weight gain could be due to the presence of estragole and anethol, which have digestive stimulant and appetizing effects (El-Sayed et al., 2015).

Table 2. Evaluation of weight parameters of control, Mn, and FEO-treated rats

<table>
<thead>
<tr>
<th>Weight</th>
<th>Organ</th>
<th>Mn</th>
<th>FEO</th>
<th>Mn+FEO</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td>96.94±0.31***</td>
<td>127.69±0.38</td>
<td>122±1.09***</td>
<td>130.4±0.40</td>
</tr>
<tr>
<td>Relative weight (g)</td>
<td>Brain</td>
<td>1.43±0.06*</td>
<td>1.60±0.006</td>
<td>1.58±0.02*</td>
<td>1.59±0.017</td>
</tr>
</tbody>
</table>

The values are expressed in average ± SEM (***: P <0.001, *: P <0.05).

Neurobehavioral Tests

*Forced Swimming Test*

Statistical analysis revealed that young rats exposed to MnCl₂ during gestation and lactation showed significant resignation, characterized by an increase in total immobility time (TIM) (P <0.001) compared to controls (Figure 1). This may be due to the fact that Mn acts as an element causing depression by acting on the monoaminergic systems especially the serotonergic system in different parts of the brain, mainly the striatum, hippocampus and pituitary-pituitary axis. This result is a good argument that confirms that rats intoxicated by MnCl₂ during the developmental period have a depressive behavior that is detected by the forced swimming test. Some authors explain this by the effect of Mn on serotonin (5-HT) which is metabolized to 5-hydroxyindoloacetic acid (5-HIAA). A decrease in serotonin has been reported in *globus pallidus* and basal ganglia (Moreno et al., 2009). Most antidepressants act by increasing the availability of 5-HT in the brain, inhibiting its reuptake and promoting its interaction with 5-HT1A and 5-HT2 receptors (Rauhuz et al., 2008). For further investigation into the effect of stress on Mn, we measured blood glucose levels in rats. Our results indicate hyperglycemia in MnCl₂ intoxicated rats compared to control rats.

![Figure 1](image_url)

**Figure 1.** The immobility time during the forced-swimming test of young control, intoxicated Mn, intoxicated and FEO-treated rats. Values are expressed as mean ± SEM; Mn vs T (***: P <0.001); Mn-FEO vs Mn (P <0.001).

On the other hand, young Mn intoxicated rats treated with FEO (0.1ml/kg) showed a significant decrease in TIM (P <0.001) compared to the intoxicated rats (Figure 1). This is due to the antidepressant effect of FEO.
The traditional use of this plant has proven its beneficial effect as an antidepressant (Abbas et al., 2019; Abbas et al., 2020). Similarly, the aromatic essential oil of *Foeniculum vulgare* has been successfully used for the management of depression (Glory et al., 2015). The results of our study using the forced swimming test are consistent with the study conducted by Jamwal et al. (2013), where a methanolic extract was used, as well as the study conducted by Perveen et al. (2017) using *Foeniculum vulgare* essential oil (Lépine et al., 2011). It was noted in one study that daily intake of *F. vulgare* seed extract 300 mg/kg body weight increases the concentration of catecholamines such as dopamine, norepinephrine and serotonin in various brain regions and lowers sodium levels in the same brain areas (Mona et al., 2010). Similarly, an earlier study showed that linolenic acid, a constituent of *F. vulgare*, increases the amount of neurotransmitters in the brain (Acar et al., 2002). The antidepressant action of *Foeniculum vulgare* seeds has been shown in a study by Abbas et al. (2020), on albino smiley moths with different concentrations (2% and 4%). These results suggest that fennel oil alone may be potential alternatives to antidepressant drugs to treat depression.

**Elevated plus maze test**

The test of the elevated plus maze showed that young rats exposed to MnCl₂ during gestation and lactation preferred to stay in closed arms and fled from open arms (Figure 2). This reflects a significantly higher level of anxiety compared to control rats. Certain physiological and psychological responses to stress are controlled by the hypothalamic-hypo-corticotropic axis and the cerebral monoaminergic systems. This anxious comportment may be explained on the one hand by the iron deficiency induced by chronic exposure to Mn (Molina et al., 2011) and on the other hand by the reduction in the amount of iron in the brain during this developmental period which caused an alteration in monoaminergic functions, particularly dopaminergic functions (Beard et al., 2002). In addition, the decrease in GABA (Gamma-aminobutyric acid) expression and the increase in its extracellular concentration following exposure to MnCl₂ may lead to a remarkable change in anxiety-related behaviour (Anderson et al., 2008). In contrast, no significant difference was noted in terms of time spent in the closed arm by the Mn-FEO animals compared to the control animals (P >0.05) (Figure 2).

![Figure 2](image-url)

**Figure 2.** The residence time in the Closed Arms during the Elevated Plus-Maze test of young control, Mn, intoxicated and FEO-treated rats. The values are expressed in average ± SEM; Mn vs T (*: P <0.05); Mn-FEO vs Mn (*: P <0.05).

**Open Field Test**

This test evaluates the animal’s reaction to a new and special environment, as well as its desire to explore spaces. In the open field test, it was initially observed that MnCl₂ intoxicated rats showed a significant stress state in a new environment resulting in a significant (P <0.05) increase in latency time compared to control rats (Figure 3). Some authors reported similar results (Moreno et al., 2009). In addition, animals exposed to Mn showed a significant decrease in the number of tiles crossed and in vertical locomotor activity, indicating locomotor hypoactivity compared to control rats. These results are consistent with those of the different authors (Oszlánczi et al., 2010; Molina et al., 2011; Marreilha et al., 2012).
Figure 3. Comparison of the different parameters of the Open-field test between young control, Mn intoxicated, Mn intoxicated and FEO-treated rats. Values are expressed as mean ± SEM; Mn vs T (*: P <0.05); Mn-FEO vs Mn (*: P <0.05).

MnCl₂ interacts with the GABAergic, glutamatergic and catecholaminergic neurotransmission systems. Indeed, increased degeneration of dopaminergic neurons causes dysfunction in the striatum, so it has been suggested that Mn is transported by dopaminergic neurons via the dopamine transporter (DAT) (Fitsanakis et al., 2006). These results indicate that loss of striatal dopamine resulted in inactivation of D1 receptors and a significant activation of D2 receptors (Bagga et al., 2012). D4 receptors are involved in vertical activity behaviour (Saldivar-Gonzalez et al., 2009). The imbalance favors over activation of the indirect pathway of globus pallidus neurons and excessive inhibition of the thalamus and brain stem regions (Bagga et al., 2012). On the other hand, vertical locomotor activity can also assess the animal’s emotional state. Indeed, some studies have shown that Mn targets the different mechanisms of certain cholinergic synapses and thus significantly affects the synaptic transport of astrocytes and proteins involved in the transport of acetylcholine not only in the central nervous system such as cortical regions, hippocampus, frontal cortex and parietal cortex but also in the peripheral nervous system such as nerves and muscle neural junctions (Marreilha et al., 2012). Some studies, which have evaluated the anti-stress effect of *Foeniculum vulgare*, showed that treatment reduces stress. The administration of FEO to rats previously intoxicated by MnCl₂ leads to a significant decrease in horizontal and vertical motor activity compared to control rats. This hypoactivity is mainly due to the sedative effect of fennel (Koppula et al., 2013).

The whole plant extract of *F. vulgare* showed a noticeable anti-stress effect against the stress induced by the forced swimming of the test animals. The plant extract (50, 100 and 200 mg/kg body weight) showed a significant improvement in the tested animals compared to the control group. The whole plant extract of *F. vulgare* acts as an anti-stress agent (Koppula et al., 2013). In addition, treatment with *F. vulgare* essential oil improves locomotor activity (Rao et al., 2002). It has been shown that α-linolenic acid, an important polyunsaturated fatty acid found in *F. vulgare*, has a role in learning and memory and may play a basic role in maintaining dopamine and norepinephrine levels in brain areas related to recall and cognitive abilities, such as the cerebral cortex, hippocampus and striatum (Joseph et al., 2005). Anxiety is the unpleasant feeling of fear and worry. If anxiety becomes excessive, it can be considered a disorder of anxiety. The fennel is an anxiolytic drug used to treat anxiety and its psychological and physical symptoms. Naga Kishore et al. (2012) studied the anxiolytic activity of the ethanolic extract of the fruit of *F. vulgare* using maze and open field test models. The dose of 100 to 200 mg of extract per kg of body weight of the animal revealed a significant activity compared to the reference diazepam (1 mg/kg). Fennel extract may possess anxiolytic activity supporting its traditional claim for anxiolytic activity.

The effect of Mn and FEO on biochemical and histological parameters

The results show that the concentration of manganese in the brain is significantly high (P <0.05) in the poisoned rats compared to the control rats (Table 3), possibly due to associations between both prenatal and postnatal
manganese exposure and embryo-fetal toxicity (Krachler et al., 1999). In addition, studies by Li et al. (2006), indicated that manganese shares the same haemostatic regulatory voice as iron and calcium within the erythroid, duodenal mucosa, renal tissues and both blood-brain barriers and cerebrospinal fluid by accumulating and decreasing their levels within these tissues. In addition, in this study, the administration of FEO in intoxicated rats showed a considerable reduction in MnCl₂ levels in the brain.

**Table 3.** Mn levels and cerebral PAL activity in control, intoxicated and intoxicated rats treated with FEO

<table>
<thead>
<tr>
<th>Brain concentration</th>
<th>Mn (µg/g)</th>
<th>FEO</th>
<th>Mn-FEO</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn (µg/g)</td>
<td>7.17±0.24*</td>
<td>0.87±0.71</td>
<td>3.99±0.13*</td>
<td>0.89±0.12</td>
</tr>
<tr>
<td>PAL (µg p-nitrophenol/mg protein/minute)</td>
<td>1.91±0.31*</td>
<td>3.31±0.36</td>
<td>2.21±0.54*</td>
<td>3.77±0.42</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; *P < 0.05.

At the cerebral level, an experiment has been carried out to determine the oxidative status following Mn intoxication during gestation and lactation. The analysis of the antioxidant status indicated that Mn decreases the enzymatic activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), leading to a dysfunction of the antioxidant defense system (Table 4). Bhuvaneswari et al. (2013), show that high-dose exposure to Mn induces oxidative damage leading to altered activity of the enzymes SOD, CAT, GPx, and the expression of Mn-SOD and GPx genes.

**Table 4.** Cerebral antioxidant enzyme activity (SOD, GPx, CAT) in control, Mn intoxicated, Mn intoxicated and FEO-treated rats

<table>
<thead>
<tr>
<th>Brain concentration</th>
<th>Mn (U/mg of protein)</th>
<th>FEO</th>
<th>Mn-FEO</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg of protein)</td>
<td>2.51±0.26***</td>
<td>6.95±0.71</td>
<td>4.62±0.34***</td>
<td>6.98 ±0.48</td>
</tr>
<tr>
<td>GPx (U/mg of protein)</td>
<td>10.72±0.31*</td>
<td>20.62±0.61</td>
<td>15.92±0.11*</td>
<td>19.74±0.17*</td>
</tr>
<tr>
<td>CAT (U/mg of protein)</td>
<td>0.49±0.75*</td>
<td>0.81±0.27*</td>
<td>0.59±0.93*</td>
<td>0.79±0.44*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; ****P < 0.001, *P < 0.05

It is suggested that the first step in the production of ROS is the production of O₂⁻⁻ which can be converted to H₂O₂ by Mn and Cu/Zn superoxide dismutase in the mitochondria and cytoplasm. H₂O₂ can be further converted to OH in the presence of Mn or other transition metals (Martinez-Finley et al., 2013). Mn²⁺ interferes with the homeostasis of Ca²⁺ in the mitochondria by occupying the Ca²⁺ binding sites with the generation of oxidative stress, and leading to the induction of a process called mitochondrial permeability transition. The opening of a permeability transition port leads to the solubility of the mitochondrial membrane for ions and protons that cause the rapid swelling and ultrastructure change associated with the loss of the mitochondrial potential of the inner membrane, altered oxidative phosphorylation, ATP synthesis (Martinez-Finley et al., 2013). Oxidative stress is defined as a pronounced imbalance between the antioxidant and oxidizing elements in favor of the latter and their harmful effects. The origins of oxidative stress are multiple and result from the formation of reactive oxygen species (ROS) in the body (De Moffarts et al., 2005). In addition, the evaluation of the toxicity of Mn was expressed by the assay of antioxidant enzymes, namely CAT, GPx, SOD, knowing that these antioxidant enzymes help maintain the homeostasis of redox potential (Chou et al., 2013).

Antioxidants of natural origin can be used to protect humans from damage caused by oxidative stress (Scalbert et al., 2005). Fennel was known as an excellent source of natural antioxidants and contributed to the daily antioxidant diet (Shahat et al., 2011). Wild fennel has shown free radical scavenging activity with higher phenolic and flavonoid content than medicinal and edible fennel (Faudale et al., 2008).

Extensive research has shown that anethol, a compound found in *Foeniculum vulgare* has shown antioxidant potential (Aggarwal et al., 2008). Anethole has an antioxidant effect by inhibiting lipid peroxidation and free radical scavenging. In addition, it also has an inhibitory action on monoamine oxidase B, thus preventing monoamine degradation and decreasing oxidative stress (Roby et al., 2013).

To complete the biochemical analyses, we performed a histological study on the cerebellum and cerebral cortex of intoxicated, control and intoxicated rats treated with FEO (Figure 4 and Figure 5). This study revealed a very marked impairment with alterations and dissociation of the Purkinje cell layer. We also observed more remarkable cell destruction with enlarged vascular spaces and cells of different shape and size in Mn exposed rats compared to control rats.
Figure 04. Photo microscopic examination of cerebral cortex tissue stained with hematoxylin and eosin G:(x40). (A) Sections of the cerebral cortex of control and FEO-treated rats (D) appeared with normal architecture; (B) histological sections of cerebral cortex tissue in Mn intoxicated rats (Mn), sections appeared with activation of microglia (arrows) and degeneration and necrosis of neurons (arrow); (C) histological sections of cerebral cortex tissue in Mn intoxicated and FEO treated rats.

Figure 5. Photo microscopic examination of cerebellar cortex tissue stained with hematoxylin and eosin G:(x 40). (A) Sections of the cerebellar cortex of the control rats appeared with normal architecture; (B) Histological sections of cerebellar cortex tissue in intoxicated rats (Mn), sections appeared with alterations and dissociation of the Purkinje cell layer (arrow); (C) histological sections of a tissue of the cerebellar cortex in Mn intoxicated rats treated with FEO; (D) Mn intoxicated rats treated with FEO.

Anatomical studies have shown abnormal development of Purkinje cells of the cerebellum and abnormalities of their dendritic branching in cats treated with heavy metals (Patrick et al., 1995). Neurophysiological studies by Kumar et al. (2014), have also shown a decrease in the amplitude of potentials in Purkinje cells of the cerebellum.
subjected to the action of heavy metals such as lead. And the cerebellum has an important role in motor coordination that is moderately impaired in humans and animals exposed to low doses of heavy metals (Pb) (Carlson et al., 1991).

In addition, we observed degeneration and necrosis of neurons in the cerebral cortex of intoxicated rats and the PAL-induced decrease indicated that brain function was affected. Mn has been reported to exert various effects at several sites in the central and peripheral nervous systems (Amany et al., 2015). It disrupts the function of basal ganglia as well as cortical regions of the brain, such as the hippocampus, frontal cortex and parietal cortex. In the peripheral nervous system, Mn exerts its effect on motor nerves and neuromuscular junctions (Marreilha et al., 2012). Mn has also been shown to induce neuronal degeneration of the caudate nucleus, putamen, globus pallidus, cerebellum and black matter as well as morphological alterations in neurons of the frontal cortex, hippocampus, midbrain and annular protuberance (Amany et al., 2015).

Histological evaluation of the studied brain regions showed a decrease in the total number of cells and an increase in the number of damaged cells. Our results show that morphological changes in rat brains correlate with oxidative stress induced by Mn and are similar to those reported by Kumar et al. (2014). Neuronal injury following exposure to Mn is limited to the cerebral cortex and cerebellum. This neuronal degeneration has been previously reported in some in vivo experiments as reported by Finkelstein et al. (2007). Administration of the essential oil of *Foeniculum vulgare* in intoxicated rats indicated that the treatment significantly improved brain architecture, to some extent, altered brain histopathology. In addition, treatment with the essential oil of *F. vulgare* offers a neuroprotective effect (Rao et al., 2002).

**CONCLUSION**

Exposure of Wistar rats during gestation and lactation to Mn revealed neurobehavioral disorders as well as neurotoxic effects that are reflected in a significant alteration of the antiradical system represented by the different enzymes. Treatment with essential oil of *Foeniculum vulgare* in rats previously intoxicated leads to a rehabilitation of this system and correction of these disorders.

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**Conflicts of Interest**

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

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