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Evaluation of Some Neurotransmitters and Oxidative Stress by Manganese Chloride After Treatment with Lavender Ethanolic Extraction in Male Rats

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Abstract. *This search intended to speculate the role of lavender ethanolic extract by reducing the toxicity of manganese in male rats. 32 white male Norwegian rats were divided into 4 equal groups. Group 1: as a control group left without treatments (only 1 ml of distilled water/ animal/ day). Group 2: were dosed manganese chloride at a concentration of 100 mg/ kg b.w. / day. Animals in Group 3 and Group 4 were dosed Manganese chloride at a concentration of 100 mg/kg b.w. then lavender ethanolic extract at a concentration of 200 and 400 mg/kg respectively, all by oral gavages and treated daily for six weeks. Then the next criteria were investigated: Neurotransmitters (dopamine and Ach (acetylcholine) in the mid brain) serotonin in serum. Oxidation indicators (glutathione and malondialdehyde in the brain and superoxide dismutase and catalase in serum). The results exhibited in both groups 3 and 4 there were a significant decrease in the concentrations of Ach accompanied by a significant increase in levels of dopamine and serotonin, also return levels of MDA (Malondialdehyde) to normal, as well as a significant increase in GSH (Glutathione) concentrations in rats mid brain. Moreover, the significant rising of SOD (Superoxide dismutase) and CAT (Catalase) levels in serum of rats in these groups indicated a noticeable improvement was achieved by lavender ethanolic extraction as compared to group 2. Conclusion, the antioxidant and antitoxic activity of lavender ethanolic extract promises in grate achievement in various health fields, including medicine, food industries and cosmetics.*

Keywords *Serotonin, Lavender, Rats, Oxidative Stress*

1. INTRODUCTION

One of the essential trace element that found naturally in the body is the Mn (Manganese), is involved in several key processes, it acts as a cofactor for several cellular enzymes, e.g. arginase, pyruvate carboxylase, (SOD) superoxide dismutase, glutamine synthetase and alkaline phosphatase (Chandel & Jain, 2016). Mn deficiency in humans is rare, due to the fact that there are high levels of Manganese in a lot of food, although Mn compounds or Mn in production are widely used in the manufacture of many foods. Though, wide manufacturing use of Mn compounds or Mn in production of paints, glass ceramics, steel, fertilizers, dry cell batteries, certain pesticides, in leather industries and texture and as antiknock agent in gasoline as well as smelting operations and mining of Mn may obtained exposure of workers to immoderate amount of this metal (Santamaria, 2008). Experimental studies have shown that high doses of Mn cause critical neurological toxicities (Jiang & Zheng, 2005). It is also known that prolonged exposure to Mn results in developmental changes and impairment of reproductive function (Chandel & Jain, 2014). Neurologically manganese accumulation in the basal ganglia may alter levels of neurotransmitters, such as dopamine (DA), acetyl choline (Ach) and others in pathways postsynaptic to the nigrostriatal system (Bowman et al., 2011). which produces observed motor symptoms. In general, conflicting

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reports regarding the results of exposition to manganese on neurochemistry and motor function are available from animal studies. Lavender oil is an important essential oil with many applications. The essential oil of lavender is sold widely herbal medicine as over the counter for the treatment of the stress that caused by many toxic substances, all global organizations, inclusive (ESCOP) the European Scientific Cooperative on Phytotherapy, (WHO) the World Health Organization and (EMA) the European Medicines Agency, accepted lavender to moderates stress, restlessness and anxiety , a novel organized review has also established essential oil of L. angustifolia for the treatment of generic anxiety (Barić et al., 2018). Different studies have been conducted to understand the mechanism of action of lavender on nerve tissue. Lavender inhibits the inflammatory response of lipopolysaccharides induced in human monocytes (Huang et al., 2012). Lavender and linalool have been reported to have similar antioxidant activity and weak cholinergic inhibitions. In neuromuscular junction, linalool is inhibiting acetylcholine release and changes the activity of anion channels (Re et al., 2000). These monitorings suggest that lavender may have several objectives to treat antioxidant, neuroprotective and anticholinergic activity. Estimation of the effects of lavender extraction on motor activity and its connection with dopaminergic neurotransmission of olfactory bulbs in mice, it is apparent that an interperitoneal injection of lavender extraction has significantly increased rotarod function and promoted D3 dopamine receptors type 3 (Kim et al., 2009). Lavender is aforementioned to play an important role in antianxiety, antidepression, analgesic, and anticonvulsant effects (Hritcu et al., 2012). Two different concentrations of Lavender Essential Oil were used in this study, with a view to reversing the effects of Mncl on certain neurotansmitters and antioxidants in male rats.

2. METHODS

Animals

32 males of Norwegian rats in age approximately 2-3 months and weighing between (250 - 300 grams) were kept in a temperature-controlled environment of 22-25°C and under 12 h: 12 h light/dark cycles, with free approach to food and water. The study was approved by the Institutional Ethical Committee in the college of veterinary medicine, department of physiology, Shatrah University, Thiqar, Iraq, and all experimental protocols were conducted in accordance with the guidelines for the care and use of laboratory animals.

Plant Extraction and Chemicals

Dried aerial parts of L. angustifolia were prepared from the local market in Basra governorate, Iraq. In 24 h, they were powdered and macerated with 70% ethanol followed by

three further cycles of this process. Following each extraction, the residual material was filtered and combined solvents evaporated using a rotary evaporator for drying. The resulting extract was kept at 4 C for a period of time before being used. Manganese chloride (MnCl2) was obtained from laboratory Chemicals in college of science in Thiqar university, MnCl2 solution was prepared, used the dose 100 mg/ kg of body weight after dissolving it in distilled water then were given to each animal at 1 ml per day by oral dosage.

Experimental Design

Thirty-two male Norwegian rats were divided indiscriminate into 4 groups each of 8 rats.

Group 1: control group, wholesome rats without treatment (administrated daily with 1 ml/ animal) only distilled water by oral gavage.

Groups 2: treated with MnCl2 at the dose 100 mg / kg of body weight after dissolving it in distilled water for 6 weeks.

Group 3: treated with MnCl2 at the dose 100 mg / kg of body weight after dissolving it in distilled water and lavender 200 mg / kg and, daily administrated by oral gavage for 6 weeks

Group 4: treated with MnCl2 at the dose 100 mg / kg of body weight after dissolving it in distilled water and lavender 400 mg / kg ,, daily administrated by oral gavage for 6 weeks .Twenty-four hours after the last treatment, animals were sacrificed, drawing blood directly from the heart without anesthesia using a puncture heart, after that animals were killed by subjected to decapitation, the head was placed in an ice dissecting basin, the skull was opened, the brain was extracted and washed by using cooled normal saline solution, then dry it with filter paper, removed the cerebellum and olfactory buds, and the remaining brain were placed in dry, clean plastic containers and kept at a temperature of -80 until tests are carried out. The drawn blood was placed in dry and immaculate glass test tubes free of anticoagulant, left at laboratory temperature for 15-20 minutes, then samples were centrifuged at 3000 rotations / minute a duration of fifteen minutes in order tos separating serum, then isolating serum and preserving it at a temperature of -20°C until use.

Study Standards

Neurotransmitter Assays : Brain tissue was collected post euthanasia and analyzes for dopamine, Ach and serotonin levels were performed using the high-performance liquid chromatography (HPLC) method. Serum catalase (CAT) and malondialdehyde (MDA) were estimated by the method used by (Wang et al., 2009). The concentration of glutathione (GSH) in serum was measured by using the modified method of Sedlak (Sedlak (1968)). the method is based on the use of Ellmans reagent-5,5 (dithio bis(2-Nitrobenzoic acid) DTNB,(which

reacts with glutathione to form a colored product whose absorbance is read at 412 nm. The technique suggested by Sun et al. was used to measure SOD activity (Sun et al., 1988).

Statistical Analysis

In order to determine the difference significantly between groups, the one-way ANOVA-test was applied. Differences between data were contrasted by least significant difference (LSD). The mean standard deviation has been expressed for all data. All statistical tests were completed by using the statistical program SPSS (version 21.0) at the significacy of $p \leq 0.05$ (Bryman & Cramer, 2012).

3. RESULTS

In the present study the results in table (1) showed that levels of dopamine and serotonin were significantly decreased ($P \leq 0.05$) in animals gave Manganese Chloride in G2 as compared to control group G1. On the other hand, treatment with Lavender in groups G3 and G4 caused a significant increase in levels of these neurotransmitters as compared to G2 that were dosed with manganese chloride only. While levels of Ach in the same table were significantly increased ($P \leq 0.05$) in rats treated with manganese chloride as compared with control group. However, treatment with Lavender in groups G3 and G4 produced a significant decrease in level of Ach as compared with those in G2. The results in table (2) displayed a significant increase ($P \leq 0.05$) in the ratio of MDA in rats treated with Manganese Chloride compared to those in control group. Treatment with Lavender in groups G3 and G4 caused a significant reduction in levels of MDA as compared to those in G2. Also, the results in table (2) showed a significant decrease ($P \leq 0.05$) in GSH, SOD, CAT levels in rats treated with Manganese Chloride comparison to those of control group. On the other hand, treatment with Lavender in groups G3 and G4 led to significant increasing of GSH, SOD, CAT levels when compared to those in G2.

Table 1. The ameliorative effects of lavender on some neurotransmitters in mid brain and serum of male rats after giving MnCl2.

Groups	Dopamine (pg./ml)	Ach(pmol/L)	Serotonin(ng/g)
G1	19.21 ± 1.17 a	36.6 ± 1.14 c	433.3 ± 6.50 a
G2	6.59 ± 0.49 c	60.8 ± 3.96 a	204.64 ± 26.82 c
G3	15.63 ± 1.26 b	48 ± 4.74 b	374.22 ± 50.91 b
G4	17.83 ± 1.16 b	45.8 ± 4.49 b	404.7 ± 43.31 a

Different letters indicate the significant differences at $P \leq 0.05$.

Table 2. The ameliorative effects of lavender by decreasing the oxidative stress in mid brain and serum of male rats after giving MnCl2.

Groups	MDA(nmol/mg)	GSH(Mnol/mg)	SOD(U/ml)	CAT(U/L)
G1	0.46 ± 0.04 d	0.85 ± 0.09 a	32.90 ± 2.84 a	0.75 ±0.08 a
G2	1.42 ± 0.15 a	0.20 ± 0.03 d	15.66 ± 2.92 d	0.42 ± 0.06 d
G3	0.93 ± 0.06 b	0.54 ± 0.09 c	25.71 ± 3.04 c	0.56 ±0.07 c
G4	0.69 ± 0.06 c	0.65 ± 0.17 b	27.48 ± 1.92 b	0.59 ±0.08 b

Different letters indicate the significant differences at $P \leq 0.05$.

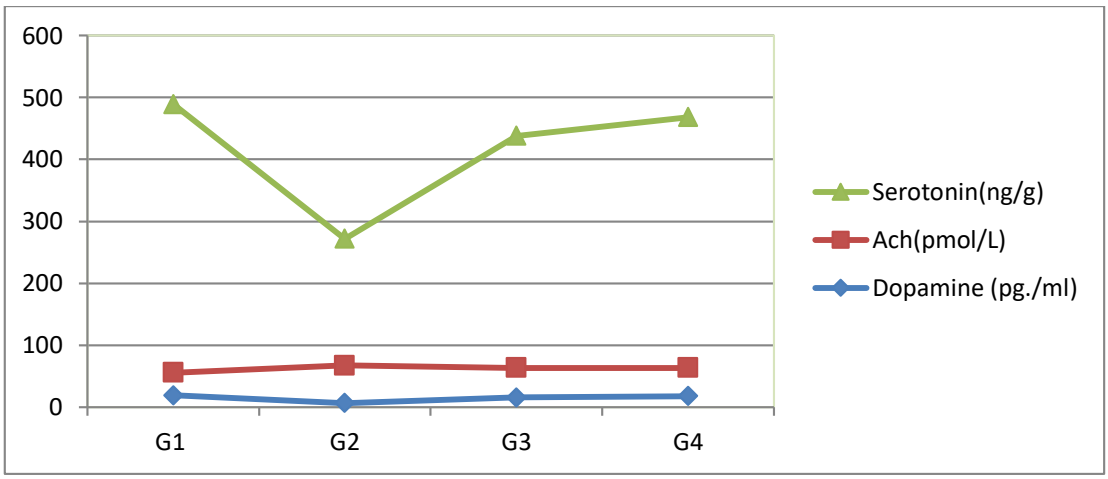

Figure 1. The ameliorative effects of lavender on some neurotransmitters in mid brain and serum of male rats after giving MnCl2.

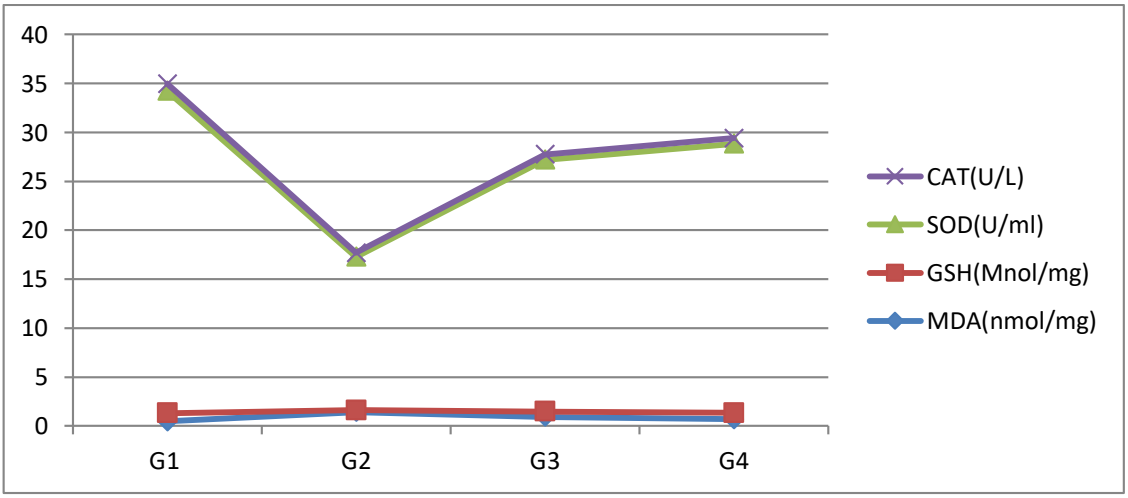

Figure 2. The ameliorative effects of lavender by decreasing the oxidative stress in mid brain and serum of male rats after giving MnCl2

4. DISCUSSIONS

Manganese (Mn) is a significant trace element that plays an important role in the protection against oxidative stress and acts as an enzymatic cofactor. Because of heavy

industrial use, smelting operations and mining a large inhabitation is chronically insecure to metal. Human health may be restricted by exposure to high concentrations of Mn (Health & Services, 1999). Lavender is utilized in pharmacy, herbal medicine, and essential oil therapy for addressing issues related to the central nervous system disturbances, such as sleep disorders , anxiety and stress (López et al., 2017). Some clinical considerations suggest that oils such as lavender exert psychological and CNS (central nervous system) influences (Perry & Perry, 2006). Several investigations were performed to clarify the mechanism of action of lavender in neuronal tissues. However, the ameliorative effects of lavender on nervous system in cases of toxicity are unwell comprehend. To the best of overall awareness, a few numbers of studies have been carried out on these effects. The existing study was assumed to consideration the ameliorative effects of two different doses of lavender on some neurotransmitters and antioxidants after dosage of 100 mg/ kg b.wt of MnCl2 in male Norwegian rats. In this search levels of dopamine in the brain of rats treated with Manganese Chloride compared to control were corresponded with the findings of many studies (Martinez-Finley et al., 2013). This result may due to effect of Manganese through several mechanisms on the level of dopamine, Manganese causes inhibition of the activity of the enzyme (tyrosine hydroxylase or (T h)) that is important in the process of dopamine building and this has been confirmed by researcher (Zhang et al., 2003). who noticed that treatment with manganese suppresses the expression of this gene leading to decrease production of dopamine. As well as the direct effect of Manganese on the central nervous system (Volken et al., 2001). Manganese accumulates in the brain, especially in areas rich in dopamine, such as the autonomic ganglia, causing their cells damage (Guilarte et al., 2008). Also Manganese transports in the brain through calcium channels, including Voltage –Gated Calcium Channels (VGCC), it was observed that the gene expression for VGCC in dopaminergic cells is greater than in non-dopaminergic cells, and this explains the increase accumulation of manganese in dopaminergic cells (Dukhande et al., 2006).On the other hand the dopaminergic ameliorative effects of lavender in this search may due to that lavender significantly elevated activity and reinforced the dopaminergic receptors in the brain which called subtype D3, these results were matched with (Hritcu et al., 2012).who evaluated that the lavender oil has impact on the activity of mid brain in mice, as well as he proved there is a relationship between the lavender and the dopaminergic receptors in the olfactory bulbs of the same animals . The significant increase in (Ach) levels of rats treated with manganese chloride may be attributed to the effect of manganese on the metabolism of Ach through inhibiting the activity of the enzyme acetylcholin esterase. (AchE) (Fitsanakis et al., 2008).is mainly responsible for the degradation of Ach and termination of the cholinergic response at

nicotinic Ach receptors. And muscarinic in the brain (Tuschl et al., 2012). which results in Ach not being degraded and thus increasing its level in the brain. This was confirmed by the researcher of (Sato et al., 2010).who pointed to that the manganese inhibits the activity of the AchE enzyme and thus reduces the decomposition of Ach, leading to its increasing and accumulation in the synaptic cleft leading to stimulation of nicotinic and muscarinic receptors. The ameliorative effects of lavender on Ach levels in groups G3 and G4 in this search are matched with several inspections were completed to demonstrate of lavender mechanism of action in nervous tissue. These effects may be attributed to the cholinergic inhibition of lavender which proved by (Wang et al., 2012). this achieved by inhibition the release of acetylcholine and alters functions of the ion channel at the neuromuscular junction as a neuroprotective activity of lavender (Re et al., 2000). The significant decrease of serotonin (5-HT) values in this search caused by MnCl were matched with the results of (Blecharz-Klin et al., 2012).Who proved the significant decrease in 5-HT levels and its metabolite in the cortex, striatum and hippocampus of rats administered Manganese by intranasal application. Also were matched with the reduction of 5-HT levels that observed in the rats frontal cortex after intraperitoneal injection of Mn (Bouabid et al., 2014). These effects may cause by the modulation of the serotonergic neurons occurred by MnCl.

In this search the results of serotonin levels in rats midbrain treated with Lavender extraction were matched with results of (Nan Lv et al., 2013). This may explain the improval effects of lavender on serotonin levels in treated groups. It has also recently been proclaimed that essential oils can potentially function as antidepressants, as they have been found to impact neurotransmitter pathways, particularly in the serotonin system. Malondialdehyde (MDA) formation is mostly used as the indicator of lipid peroxidation. Oxidative damage to membrane lipids in the brain may causes by Free radicals (Cordiano et al., 2023). In this search, the significant elevation in (MDA) levels with a significant dropping in glutathione level (GSH) in the brain tissue of rats after treatment with manganese chloride compared to control may consistent with the findings of other studies (Farina et al., 2013). but it does not with what was mentioned by (Zhang et al., 2008). These results are also consistent with a study of (Ihsan R. Ibrahim, (2015)). which indicated elevation of MDA and lowering of GSH levels in the brain of rats when treated orally with Manganese chloride at a concentration of (150m mol/kg) for four weeks. The reason for this result may be due to the oxidative stress caused by manganese, also that mentioned by the study of (Diederich et al., 2012).who indicated a decrease in the ratio of GSH with an elevation of the value of rat brain uric acid when dosed a Manganese chloride at a concentration of 200 mg/ kg of body weight for three months. The reason for all

this disturbance was explained by the fact that Manganese stimulates oxygenation by ROS (Ihsan R. Ibrahim, (2015)). Levels of MDA and GSH in G3 and G4 may indicate the antioxidant activity of different concentrations of Lavender extract. The statistical analysis revealed a significant lowering in the grade of the enzymes SOD and CAT in serums of the treated animals with Manganese Chloride for both study periods compared to control, this results agreed with what mentioned by (Martin et al., 2008). This decrease may be attributed to the increased generation of free radicals caused by oxidative stress resulting from manganese (Assessment, (2009)).which leads to the rapid exhaustion of antioxidant defense systems that perform their work by removing free radicals, thus reducing their level in the blood serum. The SOD enzyme protects tissues from damage by reactive oxygen species (ROS) by converting the super oxide radical into peroxide hydrogen H2O2 (Chis et al., 2009). while the CAT enzyme is responsible for the conversion of H2O2 to water molecule (Andringa et al., 2008). On the other hand, Lavender prevented the rise in MDA levels and caused elevation of GSH, SOD and CAT levels in treated groups G3 and G4. This may due to lavender extraction possess antioxidant activity represented by the phenolic acids, which contribute to its bioactivity. Lavender phenolic compounds have been originally investigated for their antioxidant effects, confirming a positive correlation of content of antioxidant levels to phenolic acids. This is in convention with the observations that lavender oil owned antioxidant and anti-inflammatory effects (Hancianu et al., 2013).Through intensive research, it has been approved that lavender extract shows potent detoxifying features, constructively neutralizing deleterious substances and conserving cells against oxidative damage caused by free radicals. However, further studies are ensured to clarify its optimize extraction methods and determine its efficacy and long-term safety. Overall, the confirmations presented substantially support the favorable role of lavender ethanolic extract as a beneficial therapeutic agent in enhancing health and improving well-being.

5. CONCLUSION

The antitoxic and antioxidant activity of the ethanolic extract of lavender is promises in grate achievement in various health fields, including medicine, food industries and cosmetics.

6. ACKNOWLEDGEMENT

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