Pharmacogn. Mag.
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ORIGINAL ARTICLE

Neuroprotective Effect of the Essential Oil of *Lavandula officinalis* against Hydrogen Peroxide-induced Toxicity in Mice

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Submitted: 11-Dec-2019 Revised: 21-Jan-2020 Accepted: 21-Apr-2020 Published: 20-Oct-2020

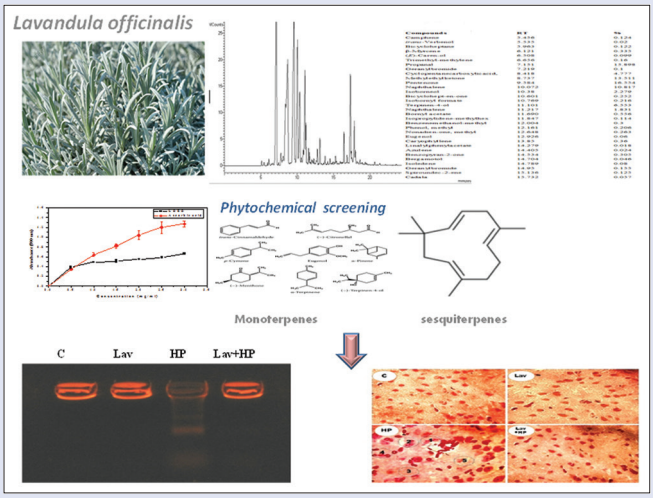
ABSTRACT

Background: Brain is the main organ that manages all other organs and has significant oxygen requirements, which makes it vulnerable to reactive oxygen species, thus causing different neurological disorders. The aim of this work is the evaluation of *Lavandula officinalis* essential oil neuroprotective effect against hydrogen peroxide (HP)-induced toxicity in mice. **Materials and Methods:** Essential oil was extracted by hydrodistillation using a Clevenger-type apparatus. Essential oil analysis is performed using gas chromatography-mass spectrometry (GC-MS). Biological activity evaluation carried out the ferric reducing antioxidant power test, deoxyribonucleic acid (DNA) fragmentation assay, the histopathological study of the brain, and determination of antioxidant enzyme activities. **Results:** Chemical characterization of essential oil using GC-MS identified 47 compounds, accounting for almost 80% of the total oil and indicates the occurrence of monoterpenes and sesquiterpenes. The identified major compounds are pentanone (16.55%), propanal (15.89%), methyl ethyl ketone (13.51%), naphthalene (10.81%), terpinen-4-ol (6.55%), cyclopentanecarboxylic acid (4.77%), and isoborneol (2.27%). This study allowed us to investigate the effects of HP on brain function in *Mus musculus* adult mice by assessing DNA degradation, cell morphology, oxidative balance and brain weight variation. Furthermore, we have highlighted the beneficial effects of *L. officinalis* essential oil, which could significantly counteract all these alterations by its active compounds, which are endowed with potent biological activities. **Conclusion:** We can conclude that HP-induced damage in histomorphological changes in mice brain, significant atrophy, as well as an important alteration of the genetic expression.

Key words: Brain, DNA, essential oil, *Lavandula officinalis*, oxidative stress

SUMMARY

- *Lavandula officinalis* essential oil has neuroprotective effect against hydrogen peroxide (HP) induced toxicity in mice
- Gas chromatography-mass spectrometry analysis of essential oil identified 47 compounds, mainly mono and sesquiterpenes as major constituents
- This study allowed to investigate the effects of HP on brain function in *Mus musculus* adult mice.



Abbreviations used: CAT: Catalase; DNA: Deoxyribonucleic acid; $FeCl_3$: Ferric chloride; FRAP: Ferric reducing antioxidant power; GABA: Gama-aminobutiric acid; GC-MS: Gas chromatography-mass spectrometry; GPx: Glutathione peroxidase; H_2O_2: Hydrogen peroxide; HP: Hydrogen peroxide; $K_3Fe(CN)_6$: Potassium ferricyanide; MDA: Malondialdehyde; NBT: Nitrobluetetrazolium; ROs: Reactive oxygen species; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances; TBS: Tris-Buffered-Saline; UI: International units.

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DOI: 10.4103/pm.pm_460_19

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INTRODUCTION

During the last decades, the increasing use of natural and synthetic substances, thus causing adverse effects on human health, led scientists to seek effective solutions. In particular, peroxidized compounds have attracted a lot of attention because they induce several damages.[1] In this context, the use of hydrogen peroxide (HP) in many areas implies that a lot of people around the world have been receiving higher or lower doses of HP. In fact, HP is used such as oxidizing agent with a wide number of industrial applications, for example in wood pulp, hair, and cosmetics, as a disinfectant and neutralizing agent in wine distillation,[2] in glass bottles cleaning, in the hospital environment (mixture with per acetic acid), in the swimming pool water treatment industry (in replacement of chlorinated products), in the food and juice processing and packaging.[3]

Cite this article as: Akermi S, Almeida JR, Khedher A, Bouzenna H, Dhibia S, Feriani A, *et al*. Neuroprotective effect of the essential oil of *Lavandula officinalis* against hydrogen peroxide-induced toxicity in mice. Phcog Mag 2020;16:464-70.

Moreover, peroxidic compounds administered by different routes have been shown to cause adverse effects inducing then certain neuronal damage.[4] It is therefore important to detect the potential toxicity of these substances. The brain plays a vital role in the control and functioning of almost all our organs that are influenced by exogenous agents, and it has become clear that these substances may act directly or indirectly on the neuronal tissue to produce a variety of biological effects of both physiological and pathological significance.[5]

Scientists have proven that despite HP's involvement in many biological processes, repeated and uncontrolled use can be a cause of oxidative stress. This causes consequently, many disorders.[6] Indeed, several antioxidants have been proved efficient in protecting some affection against HP treatment deleterious effect.[7] Many efforts have been made by researchers to implicated strategies to prevent or treat chemical-induced complications, among which we can quote phytotherapy, which is the use of a plant or one of its active ingredients to oppose more or less serious complications.[8]

Lavender (*Lavandula officinalis*) has proved successful in protecting different organs against oxidative stress in various situations.[9,10] It is an aromaic plant of the "*Lamiaceae*" family from the Western Mediterranean basin. It is not only widely used as traditional medicine, but also known to possess important biological properties.[11] The essential oil extracted from the leaves of *L. officinalis* has been reported to possess antioxidant, anti-inflammatory, antimicrobial, antioxidant, and hypnotic effects.[12] It has also been demonstrated that the efficiency of this oil is attributed to the active plant components. However, the therapeutic effect of *L. officinalis* against HP-induced neurotoxicity has not been studied yet.

In this context, the present study was conducted to explore the protective effect of Lavender essential oil on neuronal damage induced by HP in mice.

MATERIALS AND METHODS

Plant material

L. officinalis leaves were collected from a rural area around Gafsa (34.4311°N, 8.7757°E, Tunisia) in spring. A voucher specimen (n° LO02) was deposited at the herbarium of the Faculty of Sciences of Gafsa, University of Gafsa. The plant material was dried, ground using a blender, and subjected to hydrodistillation.

Extraction of essential oil

Essential oils were extracted by hydrodistillation of dried plant material (50 g of Lavender in 500 ml of distilled water) using a Clevenger-type apparatus for 5 h, as described in the European Pharmacopeia (NIST/EPA/NIH Mass Spectral Library with Search Program: Data Version, NIST 08). We performed repetitions to have enough quantity for our tests. The essential oil was collected, dried under anhydrous sodium sulfate, and stored at 4°C until future use.

Analysis of the essential oils by gas chromatography-mass spectrometry

The essential oils were analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 220 and 290°C, respectively. The column temperature was programmed from 80 to 220°C at a rate of 4°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gas (Helium) was 1.0 ml/min. A sample of 1.0 µl was injected, using split mode (split ratio, 1:100). Retention index comparison was used to identify the components, relative to *n*-alkane index and gas chromatography-mass spectrometry (GC-MS) spectra from a home-made library, constructed based on the analysis of reference oils, laboratory-synthesized components and commercially available standards.

Ferric reducing antioxidant power test

The reductive power test consists of evaluating the ability of the sample to give an iron-converting electron of Fe^{3+} form to Fe^{2+} form, which can be quantified by measuring the blue color formation at 700 nm. Therefore, a high absorbance indicates that the sample has great reducing power. One millilitre of a phosphate buffer solution (0.2 M, pH = 6.6) and 1 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] are added to 0.5 ml of the different concentrations of the samples prepared in DMSO. After incubation for 20 min in a water bath at 50°C., 1 ml of a 10% TCA solution is added to the reaction medium. After centrifugation for 10 min at 3000 rpm, 1.5 ml of the water and 100 µl of 0.1% ferric chloride are added to 1.5 ml of the supernatant. The absorbance is determined at 700 nm against a blank containing all the reagents in the absence of the sample tested.

Experimental design of animals

A total of 24 *Mus musculus* adult female mice, approximately 37 g body weight, obtained from the animal production unit, Sfax, were maintained for a 2-week adaptation period under the same conditions of temperature (22°C ± 2°C), relative humidity (50% ± 4%) and a constant photoperiod (12 h light/dark cycle). Animals were fed pellets (SNA, Sfax, Tunisia). After the adaptation period, the animals were placed in four groups, with six mice in each group. Before starting any type of treatment, we have carried out toxicity tests for the plant as well as the HP treatment was then administered as follows: Group I (C): that received vehicle only (olive oil) and considered as the normal control group.

Group II (Lav): that received essential oil of *L. officinalis* diluted in the olive oil (200 mg/kg body weight).[12]

Group III (HP): that received daily HP dissolved in olive oil at a dose of 774 mg/kg body weight/day.[13]

Group IV (HP + Lav): that received essential oil of *L. officinalis* and, at the same time, treated with the HP.

The treatment was orally (gavage) for four successive weeks. Mice of the 2nd and 4th groups were treated with essential oil of *L. officinalis* for 14 days before HP administration.

Animals from each group were sacrificed rapidly by decapitation to avoid the effect of stress. The brains were removed, cleaned, and weighed to study the variation of their weights and finally stored at −80°C until use.

Animal ethics statement

The animals were treated in accordance with the Tunisian code of practice for the care and use of animals for scientific purposes as well as the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe NO123, Strasbourg, 1985).

Deoxyribonucleic acid fragmentation assay

Deoxyribonucleic acid (DNA) samples in the brain tissue required for the DNA fragmentation analysis of normal and experimental mice were isolated using the method previously reported.[14] The DNA fragmentation assay was performed by electrophoresing genomic DNA samples on agarose/EtBr gel following the procedure previously described.[15]

Histopathological study of the brain

To study the cellular architecture, we conducted to dip parts of experimental mice's brain for 48 h into a fixative solution (10% formalin neutral buffer solution). First, we wash trimmed tissues with tap water, then dehydrated them through a graded alcohol series and passed them through xylol and paraffin series before embedding them in paraffin. Blocks were cuted into 5–6 µm sections using a microtome, stained in hematoxylin and eosin, and finally examined under the optical microscope.

Determination of antioxidant enzyme activities

Small pieces of the brain (1 g) were immersed in 2 ml ice-cold Tris-Buffered-Saline (pH 7.4) and then centrifuged (5000 ×*g*, 30 min, 4°C). Supernatants were collected and then used for determining the levels of lipid peroxidation (Thiobarbituric acid reactive substances [TBARS]) and also the variation of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) as following:

TBARS: 175 µl of 20% trichloracetic acid containing 1% butyl hydroxytoluene were added to 125 µl of supernatant. Lipid peroxidation was evaluated by measuring the substances which react with thiobarbituric acid (TBARS) containing aldehydes (including malondialdehyde) and lipid hydroperoxides, according to Yagi.[16]

SOD: we have determined the total SOD by measuring its ability to inhibit the photoreduction of nitrobluetetrazolium (NBT), referring to the method of Misra and Fridovich.[17] One unit of SOD represents the amount inhibiting the photoreduction of NBT by 50%. The activity was expressed as units/mg of proteins at 25°C.

GPx: 1 µmol of reduced glutathione peroxide oxided by hydrogen peroxide (H_2O_2) (1 min, pH 7.4 at 25°C) corresponds to one unit of GPx according to the method of Flohel and Gunzler.[18]

CAT: we have used the method of Aebi.[19] One millilitre of the reaction mixture contained 100 mM of phosphate buffer (pH 7.0), 100 mM H_2O_2, and 20 µl of the brain (=1.5 mg of protein). We have measured the decrease of absorbance at 240 nm for 1 min that refers to the H_2O_2 decomposition. We used an extinction coefficient of 0.043/mM/cm to calculate the enzyme coefficient that is expressed in International Units, i.e. in µmoles H_2O_2 destroyed/min/mg of proteins.

Statistical analyses

Data were expressed as mean, standard deviation (SD). Statistical significance was determined using one-way ANOVA, followed by a Tukey's *post hoc* test. $P < 0.05$ was considered statistically significant.

RESULTS

The chemical characterization of the essential oil using GC-MS identified 47 compounds, accounting almost 80% of the total oil and indicates the occurrence of monoterpenes and sesquiterpenes. The identified major compounds were pentanone (16.55%), propanal (15.89%), methyl ethyl ketone (13.51%), naphthalene (10.81%), terpinen-4-ol (6.55%), cyclopentanecarboxylic acid (4.77%) and isoborneol (2.27%). Table 1 shows the identified constituents, their retention time, and the corresponding amount.

The chromatogram, as shown in Figure 1, illustrates the major compounds found in *L. officinalis* essential oil.

As can be clearly seen in Figure 2, the essential oil of *L. officinalis* was able to reduce Fe^{3+} to Fe^{2+} at different concentrations ranges. The reducing power of this natural product increased with increasing concentrations and was able to serve as an electron donor. The reducing power of Lavender's oil at 3 mg/ml was found to be 0.66 ± 0.15 and thus, significantly lower than that of ascorbic acid, which was used as the positive control (1.27 ± 0.37), at the same concentration.

Table 1: Chemical composition of the essential oil of *Lavandula officinalis*

Compounds	RT	AU
Camphene	5.456	0.124
trans-verbenol	5.535	0.02
Bicycloheptane	5.963	0.122
β-Myrcene	6.121	0.335
(*E*)-Caren-ol	6.508	0.099
Trimethyl-methylene	6.656	0.16
Propanal	7.151	15.898
Geranylbromide	7.219	0.1
Cyclopentanecarboxylicacid	8.418	4.777
Methylethylketone	8.737	13.511
Pentenone	9.584	16.554
Naphthalene	10.072	10.817
Isoborneol	10.38	2.279
Bicyclohept-en-one	10.601	0.252
Isobornyl formate	10.769	0.216
Terpinen-4-ol	11.101	6.553
Naphthalene	11.217	1.831
Bornyl acetate	11.690	0.556
Isopropylidene-methylhex	11.847	0.114
Benzenemethanol-methyl	12.004	0.3
Phenol, methyl	12.161	0.206
Nonadien-one, methyl	12.648	0.263
Eugenol	12.926	0.06
Caryophyllene	13.85	0.36
Linalylphenylacetate	14.279	0.018
Azulene	14.405	0.024
Benzopyran-2-one	14.534	0.305
Bergamotol	14.704	0.046
Isoledene	14.789	0.08
Geranylbromide	14.95	0.155
Spiroundec-2-ene	15.136	0.125
Cadala	15.732	0.057
Caryophyllene oxide	15.915	0.139
Cyclopropa[*g*]benzofuran	16.438	1.53
Globulol	16.725	0.044
Cubenol	16.848	0.544
2-(4α,8-Dimethyl	17.512	0.376
Murolan	17.631	0.05
Isoaromadendrene epoxide	17.754	0.326
Lup-ene-3,2,1-dione	17.911	0.19
Eicosanol	18.912	0.08
Isopropyl myristate	19.349	0.111
Isopropyl palmitate	21.752	0.011
Benzene dicarboxylic acid	27.368	0.018
Docosene	27.688	0.025
Pentacontanol	28.421	0.019
Tetracosahex	29.407	0.173

RT: Retention time; AU: Amount of the component

Figure 3 illustrates the significant reduction by 44.44% in brain weight in HP-treated mice compared to control mice as well as in mice receiving lavender oil (42.31%) and in animals pretreated with lavender and then with HP (37.50%).

Figure 4 shows the qualitative changes in the integrity of the genomic DNA extracted from the brain tissues. The gel electrophoresis DNA isolated from control samples (lane 1) and Lav-treated tissues (lane 2) presented intact bands. However, a clear degradation of DNA, characterized by mixed smearing and laddering of the DNA marker of apoptosis were observed in the HP-treated group (lane 3). In the Lav+HP treated groups (lane 4), a less fragmentation nearly the same as controls was observed.

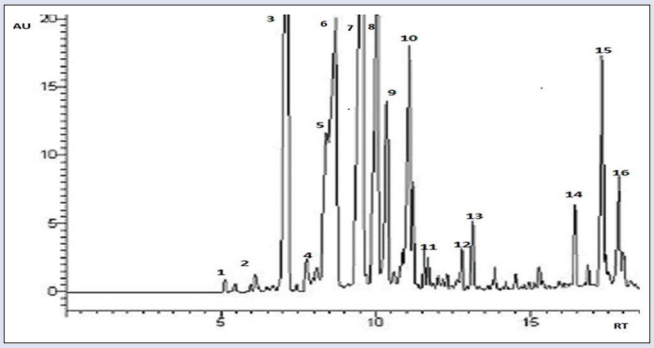

Figure 1: Gas chromatography-mass spectrometry chromatogram of the essential oil of *Lavandula officinalis*. Peaks: (1) Camphene (2) β-Myrcene (3) Propanal (4) Geranylbromide (5) Cyclopentane carboxylic acid (6) Methylethylketone (7) Pentenone (8) Naphtalene (9) Isoborneol (10) Isobornylformate (11) Terpinen-4-ol (12) Bornyl acetate (13) Caryophyllen (14) Globulol (15) Cubenol (16) Isoaromadendrene epoxide. The essential oils were analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and hydrogen peroxide -5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm)

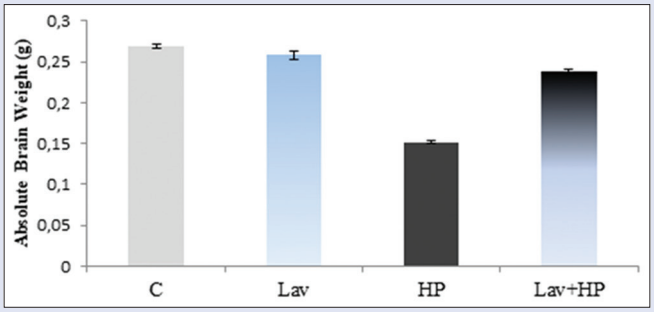

Figure 3: Variations in brain weights of mice after 6 weeks of treatments in controls (c), (Lav) mice receiving only the essential oil of *Lavandula officinalis,* (hydrogen peroxide) mice treated with hydrogen peroxide, (Lav + hydrogen peroxide) mice pretreated with Lav then treated with hydrogen peroxide. Values correspond to the mean of 6 measurements ± standard deviation. ***P* < 0.05 versus control group; ++: *P* <0.05 versus HP group

In Figure 5, we illustrate histopathological changes in the brain. The control brain has shown no microscopic lesion. HP hippocampal sections revealed remarkable damage. We notice apoptosis, congestion of blood capillary, nuclei were pyknotic, and there is chromatic condensation, neuronal atrophy, and cellular atypia. The administration of lavender to mice has significantly prevented these lesions.

TBARS concentration is a good marker of cell homeostasis. In our experimental conditions, we find that the lipoperoxidation was increased in the brain after 2 weeks of HP administration ($P < 0.05$) compared to the control group. A significant decrease was observed in the group pretreated with *L. officinalis* then receiving HP [Figure 6]. We notice a remarkable decrease in the levels of the antioxidant enzymes that protect cells from oxidative injury. In fact, CAT, SOD, and GPx were found to be reduced by (0.024; 0.119 and 0.110 UI/mg of proteins), respectively, in the tissues of mice treated only by HP compared to normal mice. These changes, which indicate an inability to counter the negative effects of HP, were corrected in mice treated with *L. officinalis*.

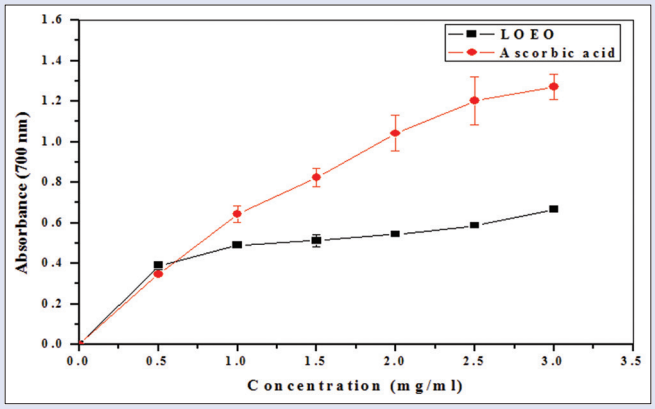

Figure 2: Reducing power of the essential oil of *Lavandula officinalis* and the synthetic antioxidant ascorbic acid at different concentrations

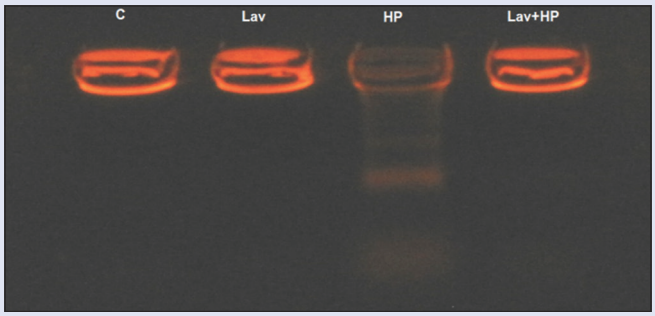

Figure 4: Agarose gel electrophoresis of deoxyribonucleic acid fragmentation. Deoxyribonucleic acid isolated from experimental brain tissues was loaded into 0.8% (w/v) agarose gels. Lane 1: Deoxyribonucleic acid isolated from control brain tissue; Lane 2: Deoxyribonucleic acid isolated from Lav treated brain samples. Lane 3: Deoxyribonucleic acid isolated from hydrogen peroxide intoxicated brain; Lane 4: Deoxyribonucleic acid isolated from Lav + HP treated brain samples

DISCUSSION

There has been a growing appreciation of the important role of oxidative stress in many diseases. The study of HP has, in fact, attracted the attention of many researchers. However, the precise nature by which HP functions as a potent agent in vertebrates has remained unknown. Neurodegenerative diseases are a major worrying situation, inviting specialists to provide effective solutions. One of the most relevant strategies used by researchers to oppose these complications is herbal medicine and more specifically, the use of essential oils. In this context, the present work was undertaken to explore the protective effects of the essential oil of *L. officinalis* on the HP-induced neuronal toxicity.

In this study, we have evidenced the effects of HP by studying the integrity of the DNA, the histological examination, the variation of the brain weight, and the study of oxidative balance. We have demonstrated that the repeated administration of HP resulted in an alteration of the expression of the DNA, an involvement of hippocampal neurons, a loss of the brain weight as well as an imbalance in the *redox* system indicating then a massive damage in neural function.

Neurons are, by nature, particularly sensitive to oxidative stress because of their high energy consumption, higher oxygen exposure, metal ion enrichment, higher polyunsaturated fatty acid composition sensitive to oxidation, and finally, their low content in antioxidant molecules.

Otherwise, peroxides are known to be able to form oxygen free radicals that are extensively implicated in several biological mechanisms.

HP and its derivatives, particularly reactive oxygen species (ROs) have significant physiological and pathological effects.[20] Proteins, lipids, and nucleic acids are targeted by oxidative reactions and damaged by free radicals.[21]

The *redox* regulation of the initiation of transcription appeared as an important element of the control of the gene expression.[22] In eukaryotes, a factor of murine transcription, activated by HP, binds to the DNA sequence recognized by OxyR.[23] A large number of eukaryotic transcription factors are sensitive to the *redox* balance in the cell; its variation really rules the activity of many transcription factors. Their degree of sensitivity is variable. The AP-1 (activator protein 1) complex is composed of a dimer that reacts quickly to various stresses;[24] it is activated *in vivo* by HP as well as ultraviolet (*via* ROs). Our findings are in agreement with Lassmann *et al.*[25] Lucassen [26] who confirm that severe brain diseases contain more cells with DNA damage and more specifically with Shimada *et al.*[27] who affirm that the DNA damage that occurs during neuronal apoptosis has been evidenced by examination of nuclear DNA strand breaks.

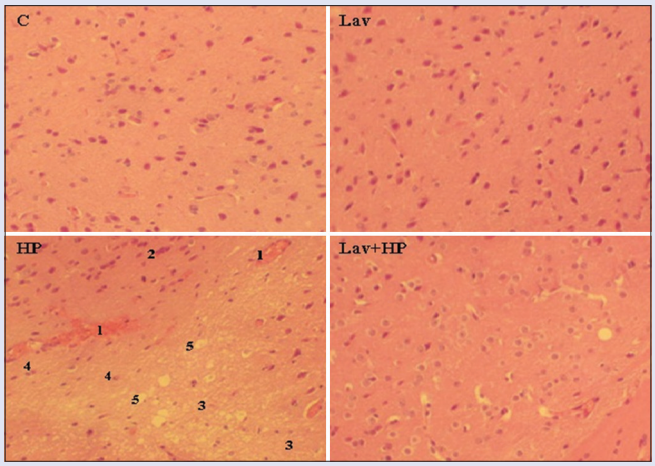

Figure 5: Histological structures of the brain of mice stained with hematoxylin eosin GX40: controls (C), receiving lavender (Lav), treated with hydrogen peroxide alone (hydrogen peroxide) and in combination with lavender (Lav + HP). 1: Congestion of blood capillary; 2: Chromatin condensation; 3: Apoptotic particles; 4: Neuronal atrophy; 5: Cellular atypia

In neurodegenerescence, the antioxidant system can no longer counter the production of free radicals ROs that accumulate in neurons and lead them slowly towards their death, especially by the mechanism of apoptosis. In fact, the alteration of the DNA is confirmed by the histological data obtained. We found significant changes in neuronal sections, such as a decrease in the total number of cells with atrophy of the cell body. The apoptotic cell appears as an isolated form, retracted with eosinophilic cytoplasm with fragments of dense nuclear chromatin. In fact, apoptosis can occur in a wide variety of situations. The death of neurons by apoptosis, common during development, also occurs when the neuron detects an abnormality in its own functioning. It is triggered by abnormalities due to the accumulation of free radicals in the cell, or by an excess of intracellular calcium due to a tear in the membrane.[28] It is an active process requiring activation signals, signal transduction, expression of genes, and protein synthesis. It can be induced or prevented by the addition or suppression of particular stimuli.[29]

We also found that there was a decrease in the relative weights of the brains of mice administered by HP. Atrophy defines a decrease in the size of an organ or tissue; this results in an imbalance between the formation of new cells not compensating apoptosis or programmed cell death. Cerebral atrophy, therefore, represents a decrease in the size of the brain that affects part or all of the brain.

According to our results, this loss of weight confirms the histological data and the genetic profile, thus testifying the induction of a neurodegenerative process. In fact, the mice brain exposed to HP showed a dose-dependent decrease which promotes atrophy of the brain as observed by[30] who demonstrates that brief exposure to HP is sufficient at clinically relevant concentrations to induce substantial

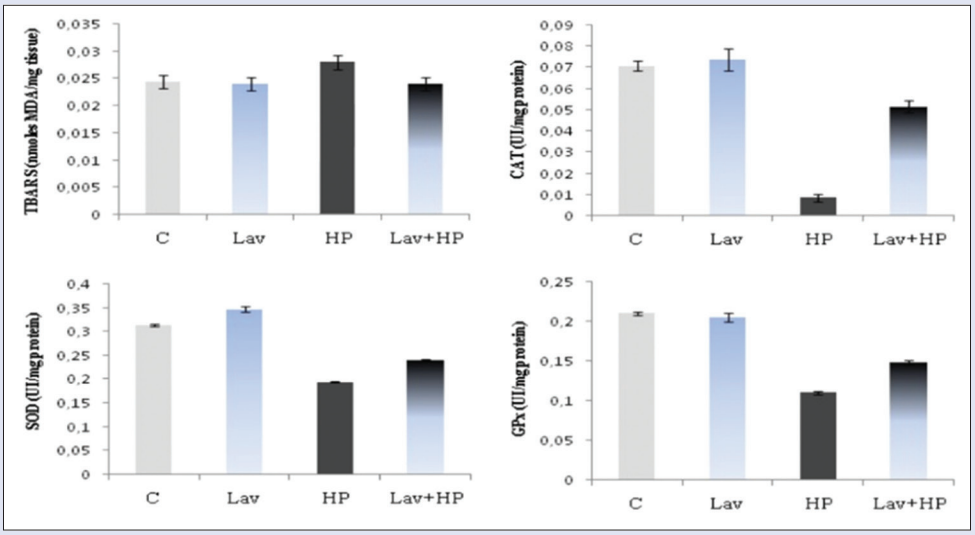

Figure 6: Levels of lipid peroxidation (expressed as thiobarbituric acid reactive substances, nmol/mg protein) and activities (international units/mg protein) of catalase, superoxide dismutase and glutathione peroxidase in brain tissues after 6 weeks of treatment in controls (c), (Lav) mice received the essential oil of *Lavandula officinalis,* (hydrogen peroxide) group given hydrogen peroxide for 2 weeks and (Lav + HP) animals pretreated with lavender then gavaged by hydrogen peroxide. Values are the mean of 6 measurements ± standard deviation. $^{+}P < 0.05$ compared to hydrogen peroxide group, $^{*}P < 0.05$ compared to control Group (c), $^{**}P < 0.01$ compared to Hydrogen peroxide group, $^{**}P < 0.01$ compared to control Group (c)

neuronal injury involving calcium channels and glutamate receptors. This is also confirmed by the work of Mesiwala *et al.*[31] who proved that 3% HP particles caused lesions following its diffusion in the human brain. Tissues have different oxygen requirements and are not evenly distributed in the body.

Although the brain accounts for only 2% of the total body weight, it consumes more than 20% of the total required oxygen.[32] Given this high demand with the abundance of highly peroxidizable substrates, the brain is therefore threatened by the deleterious effects of ROs. ROs overproduction or antioxidant systems failure results in an imbalance of cell redox state, which is called oxidative stress. Many studies have highlighted the relationship between oxidative stress and a range of neurodegenerative diseases and new approaches *in vivo* and *in vitro* propose its great impact on these diseases.[33] The neuron, as one of the main types of brain cells, is responsible for the massive consumption of oxygen and glucose. In the case of cellular stress, the partially reduced forms of oxygen are very active because the free radical is unstable and seeks to be a donor or to accept an electron. ROS interacts chemically with biological molecules because of their high reactivity resulting in structural and functional changes or even cell death. These species target different substrates, which cause the oxidation of DNA, proteins, and lipid peroxidation. To avoid the accumulation of ROs causing deleterious effects, each cell of the body has enzymatic and non-enzymatic proteins, as well as molecules of food and metabolic origin that eliminate them. SOD is the first barrier to this antioxidant defense. The existence of three different types of this enzyme whose activity and localization in the cell are complementary has been demonstrated; they ensure the elimination of oxidized anions and HP in the intracellular compartments. They catalyze the disproportionation of O_2- to HP.[34]

HP and lipid peroxides are reduced by multiple isoenzymes which belong to the family of GPx s using GSH as electron donor.[35] There are three different isoforms of GPx with different locations in the cell, and it should be noted that GPx 1 is one of the major antioxidant enzymes in the brain. It has been suggested that its regulation could be used in the prevention of neuronal damage.[36] CAT, which is located in peroxisomes and cytoplasm, is responsible for converting HP to water and oxygen.[33]

Herbal medicine helps to improve memory or delay various types of neurodegenerations. It is believed that extracts and essential oils from aromatic plants are potent candidates with their active compounds having positive effects on nerve cells.

These compounds exert a protective action against DNA damage, trap free radicals responsible for oxidative stress, protect cell membranes and provide neurotransmission.[37,38]

In this context, Zali *et al.*[39] have demonstrated the efficacy of *L. officinalis* in the treatment of the most important neurodegenerative conditions related to the massive loss of brain weight. Here, this plant was explored for its ameliorative and neuroprotective mechanism. In fact, the phytochemical screening of the essential oil of *L. officinalis* leaves revealed a complex composition with high levels of terpenic compounds, which is consistent with the work of Benyagoub *et al.*[40] with the dominance of oxygenated monoterpenes and monoterpenic hydrocarbons. These compounds attribute high pharmacologic activities to the essential oil of *L. officinalis*.[41,42] These compounds enter the brain through the blood-brain barrier nonselectively and react with the central nervous system.

The protective mechanism can be explained by its action on the antioxidant balance, calcium channel blocking[43,44] membrane stability and depolarization, vesicle trafficking, etc., the neuroprotective effects of lavender might also be related to the activities of glutamate, and gama-aminobutiric acid since these two markers are quantitatively the most important exciters and inhibitors of brain neurotransmitters in mammals[45] so that they are actively sensible to the action of lavender's constituents.[46] In addition, Mudher and Lovestone[47] proved that these monoterpenes play an important role in the activation of some protective proteins such as Snca, Hspa, Apoa1, NF-L etc…that trigger mechanisms such as anti-lipid peroxidation and anti-inflammatory effects. Besides, the Nuclear Factor NF was proved to be an important factor in the neuronal caliber that interacts with lavender since it reduces the frequency of cell death by the apoptotic process via the action of Prdx 2 and Hsp5 that were evidenced to be targeted with the major components of *L. officinalis*. Therefore, our findings showed that the essential oil of *L. officinalis* exhibited an excellent neuroprotective effect and may be considered as a useful source of cellular defense agents in the brain against HP.

CONCLUSION

HP induced damage in histomorphological changes in the brain of mice, significant atrophy, as well as an important alteration of the genetic expression. However, the essential oil of *L. officinalis* offered significant protection against the toxicity of HP by restoring these parameters to normal, that's maybe attributed to its constituents.

Financial support and sponsorship

This research was funded by the Tunisian Ministry of Higher Education and Scientific Research, Tunis, Tunisia, through the Integrated Physiology Laboratory; Macromolecular Biochemistry and Genetics. Faculty of Sciences of Gafsa, Tunisia and Laboratory of Environmental Physiopathology, Valorization of Bioactive Molecules and Mathematical Modeling, Faculty of Sciences, Sfax, Tunisia.

Conflicts of interest

There are no conflicts of interest.

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