Leaves of Lavender Protect Adult Mice from Hydrogen Peroxide-induced Injury: Evidence from *in vitro* and *in vivo* Tests

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Abstract: Medicinal plants and their secondary metabolites have long been a rich source of biologically active compounds that can prevent many diseases. In this context, we investigated the antioxidant activities of the essential oil of *Lavandula officinalis* and tested its potency against hepatic and renal toxicity induced by hydrogen peroxide in adult male mice based on measurements of biochemical parameters, oxidative stress, and tissue damage in both organs. We proved a remarkable antioxidant power of this plant (*in vitro*) by correcting the harmful effects of the prooxidant (*in vivo*). It can be concluded that lavender is an aromatic plant capable of reducing the stress caused by reactive oxygen species.

Key words: oxidative stress, biochemical parameters, antioxidant, essential oil

1 Introduction

Hydrogen Peroxide (HP) is a chemical compound that has both oxidizing and reducing properties. Its aqueous solution is colorless and slightly viscous. HP exists naturally in living beings as a byproduct of cellular respiration. Its uses have significantly grown over the last two decades. It has still remained important in water treatment, pollution control, paper industry, as a disinfectant for local treatment, and in the composition of certain dermatology treatments as well. When sprayed at high temperature, HP is used to sterilize food packaging composites just prior to the incorporation of their content (UHT liquids such as milk, fruit juice, etc). The market of this molecule is global and enormously widespread. However, HP can induce serious damage in different ways as a result of an uncontrolled exposure, therefore generating cardiovascular, neurodegenerative, gastrointestinal diseases, etc¹. These complications are a result of excessive and prolonged stress cells. One of these complications, of major concern in this study, is oxidative stress which is an assault involving biomolecules such as lipids, proteins, DNA². In order to prevent these diseases, we used natural medicines that have recently had a remarkable resurgence. In this context, experts have focused on the merits of aromatic plants by virtue of their secondary metabolites such as essential oils as a natural and rich source of powerful antioxidants, and several studies have shown that herbal drugs worldwide are an effective cure against many diseases³.

Lavender is an aromatic plant commonly used in various fields such as cosmetology, herbal medicine, aromatherapy, fragrance industry, and food manufacturing⁴. It has also been used for its sedative, antispasmodic and carminative properties⁵. Several studies have focused on the phenolic compounds and the organoleptic characteristics of this plant as well as its antibacterial⁶, antifungal⁷, antiviral and antioxidant capacities⁸,⁹. It has recently been suggested that essential oils derived from different Lavender varieties may vary in terms of their biological activities¹⁰. However, the therapeutic effect of Lavender essential oil against HP has not been well understood, and its role against free radicals generated in various organs has not been well elucidated so far. In this context, the aim of the present study was to investigate the protective effects of *Lavandula officinalis* essential oil LOEO against injurious damage of chronic doses of HP in liver and kidney functions in adult mice, by measuring biochemical parameters and exploring
the level of the oxidative stress in cells through an histopathological examination.

2 Material and Methods

2.1 Chemicals and reagents

H₂O₂, Anhydrous sodium sulphate (Na₂SO₄), Hydrochloric acid (HCl), Hexane, Sodium chloride (NaCl), Tris-buffered Saline (TBS), Thiobarbituric acid (TBA), Nitroblue tetrazolium (NBT), and Phosphate buffer (PBS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), Ascorbic acid, Vitamin E and Dimethyl sulphoxide (DMSO) were purchased from Sigma Aldrich Co (St Louis, MO).

2.2 Plant material

Leaves of Lavandula officinalis were collected during the spring season, in April 17, 2018 from the region of Gafsa in the south west of Tunisia. The Voucher specimens No.L101 have been deposited with the herbarium of the faculty of sciences of Gafsa, Tunisia (FSG). We collected 425 g of lavender, after drying at room temperature of 20°C for 7 days in shaded and ventilated space, leaves were ground into a fine powder.

2.3 Extraction and isolation of essential oil

We carried out the hydro distillation with 250 g of powder in distilled water. Then, hexane was used to collect the oil from the extraction apparatus. The organic phase was dried using Na₂SO₄ until all water traces were totally removed. After filtration, the solvent was evaporated; we recovered almost 3.25 g of the essential oil extract that was kept at 4°C in a dark glass bottle until the end of phytochemical analyses. The yield was reported per dry leaves and oil results given w/w.

2.4 DPPH free radical scavenging activity

The antioxidant potential of L. officinalis essential oil LOEO was determined by radical scavenging assays: the 2,2-diphenylpicrylhydrazyl radical (DPPH). The ability of LOEO to scavenge DPPH radical was determined using a slightly modified version of the protocol developed by Barros et al. Briefly, the DPPH solution was prepared by solubilizing 2 mg of DPPH in 100 mL of methanol, and 0.5 mL of the various standard extractors (BHT and vitamin E) were added to 1 mL DPPH. The mixture was incubated in the dark for 30 min. A control was prepared in parallel containing only the DPPH solution. The reaction mixture was incubated in a dark room for 30 min and the free radical scavenging ability was determined by measuring the absorbance at 517 nm. DPPH scavenging activity is expressed as a percentage and was calculated using the following formula:

\[ \text{% DPPH scavenging} = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100 \]

Where \( A_{\text{control}} \) is the absorbance of the control reaction and \( A_{\text{test}} \) is the absorbance of the extract reaction.

2.5 Ferric reducing antioxidant power test (FRAP)

The FRAP of LOEO was quantified by measuring the blue color formation at 700 nm. 0.5 mL of sample solution at various concentrations (from 0 to 3 mg/mL) was added 1 mL of phosphate buffer (0.2 M; pH 6.6) and then 1 mL of potassium hexacyanoferrate [K₂ Fe(CN)₆] (1%) in distilled water. The mixture was heated at 50°C in a water bath for 20 min. A volume of 1 mL of trichloroacetic acid (10%) was subsequently added and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (1 mL) was mixed with 1 mL of distilled water and 0.5 mL of freshly prepared 1% FeCl₃ in distilled water. A blank sample was prepared under the same conditions. The Ascorbic acid was used as positive control. In this method, the higher the absorbance, the higher the reduction power.

2.6 Experimental protocol

2.6.1 Animals

Thirty adult female mice (Mus musculus) weighing 37 g were bred in an animal house in the Faculty of Sfax, Tunisia, which was approved by the Tunisian Ministry of Higher Education. The conditions of the animal house meet the required international standards. The animals were allowed to adapt to their new environment and kept under photoperiods (14 h light/10 h dark) at 22-23°C. They were allowed free access to food pellets obtained from the SICO, Sfax, Tunisia, and they were given access to water ad libitum. Mice were cared for under the Tunisian Code of Practice for The Care And Use of Animals for Scientific Purposes and the Experimental Protocols were approved by The Ethics Committee (Faculty of Sciences of Sfax, Tunisia) 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).

2.6.2 Experimental design

After 10 days of acclimatization, the animals were divided into five groups of six mice each. (T−) was the negative control group, administered by daily oral gavage NaCl 0.9%. (T+) was the positive control group given, by oral gavage, the infusion of green tea Camellia sinensis (66 g dry leaves per liter of tap water) for 45 days. (LOEO) consisted of mice intraperitoneally injected with the essential oil of Lavender (200 mg/kg) for 30 days and then given NaCl for 15 days. (TOX) was the group of mice treated orally by gastric intubation with HP (774 mg/kg) which was diluted in normal saline, it was administered once a day for 15 successive days. This chronic dose has been used in studies investigating acute effects of HP in...
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2.6.3 Serum markers assays

Serum glucose levels, Alanin as well as Aspartate Transaminase (ALT, AST) activities, urea and creatinine were determined using Kit Methods (Spinreact).

2.6.4 Evaluation of antioxidant status

Homogenates of livers and kidneys were used to evaluate the oxidative stress markers. Lipid peroxidation was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) as described by Niehaus and Samuelsson (1968). Superoxide dismutase activity was estimated using the method developed by Beauchamp and Fridovich (1971). The reaction mixture concentration 50 mM of liver tissue homogenates in potassium phosphate buffer (pH 7.8) was mixed with 0.1 mM EDTA, 13 mM methionine, 2 µM riboflavin, and 75 mM nitro blue tetrazolium (NBT). The absorbance was detected at 560 nm and the results were expressed as units (U) of SOD activity/mg protein. Catalase (CAT) activity was assayed spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi (1984). Glutathione peroxidase (GPx) activity was determined according to Flohe and Gunzler (1984).

2.6.5 Histopathological study of the Liver and kidney

To further support our findings, we performed histological sections of these organs by putting parts of them for 48 h into a fixative solution (10% Formalin neutral buffer solution). First, we washed 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. Paraffin blocks were cut into 5-6 µm sections using a microtome, stained in Hematoxylin and Eosin, and finally examined under a light microscope.

2.7 Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using the SPSS version 21 software. Significant differences between treatment effects were determined using one-way ANOVA, followed by Dunnett HSD Post-Hoc tests for comparisons with statistical significance of \( p < 0.05 \).

3 Results

3.1 Yield of extraction

We have obtained an essential oil extracted from the leaves of Lavandula officinalis that had a strong odor and a yield of hydro distillation 1.31% (w/w) in relation to the dry weight of the plant.

3.2 DPPH test

According to Fig. 1, we notice that an increase of the concentration of the oil leads to an increase of the anti-

3.3 FRAP test

As can be clearly seen in Fig. 2, LOEO was capable of reducing Fe\(^{3+}\) to Fe\(^{2+}\) at different concentration ranges. Its reducing power increased with increasing concentrations and was able to serve as an electron donor. At 3 mg/mL, the reducing power of LOEO was found to be 0.98 ± 0.02 and thus significantly lower than that of ascorbic acid (0.74 ± 0.01), at the same concentration.
3.4 Effects of LOEO on HP-induced toxicity in mice

3.4.1 Effects of HP and LOEO on biochemical markers

Table 1 showed that the serum levels of Glycemia, AST, and ALT in the mice treated with HP (group TOX) were significantly changed (8.58 for glucose, 467.33 and 100.16 U/L for AST and ALT) compared with those of control group (T−) (6.65, 246.66, and 69.16 U/L, respectively). The administration of the essential oil of Lavender in the (LOEO + TOX) group induced a significant decrease in the levels of the above mentioned markers (6.23, 216.0 and 61.0) compared with values of the TOX group. However, there were no significant changes in these parameters in the T+ and LOEO groups compared to the (T−) group.

Table 2 indicated a significant decrease in HP-treated mice (12.31 mmol/L and 12.21 µmol/L) in comparison with the control group (26.71 mmol/L and 59 mmol/L, respectively). These levels were significantly increased by the pretreatment of mice by the LOEO (34.68 and 50.26 U/L).

3.4.2 Effect of LOEO on oxidative stress markers

Figure 3 shows that TBARS levels, as an indicator of lipoperoxidation was increased in both liver and kidney in the TOX-treated mice (by 0.005 nmol/mg of proteins) compared to the negative control group. A significant decrease of these levels in the LOEO + TOX group occurred.

3.4.3 Effect of HP on enzymatic antioxidant status

We noticed in Table 3 that the levels of SOD, GPx and CAT were found to be significantly reduced in the liver and kidney of TOX-treated mice (0.27/0.28 UI/mg proteins, respectively) for SOD; (0.11/0.14 UI/mg proteins, respectively) for GPx and (0.041/0.055 UI/mg proteins, respectively) for CAT, compared to control. The administration of essential oil greatly corrected these changes in the animals in the LOEO + TOX group.

Table 1 Protective effect of essential oil of Lavandula officinalis essential oil on hepatic enzymes levels of HP-induced mice. The results are expressed as mean ± SEM, n = 6 mice/group.

| Parameters      | T−       | T+       | LOEO     | TOX      | LOEO+T   |
|-----------------|----------|----------|----------|----------|----------|
| Glycemia (mmol/L) | 6.65 ± 0.30 | 6.50 ± 0.26 | 6.18 ± 0.90 | 8.58 ± 0.21** | 6.23 ± 0.37* |
| AST (U/L)       | 246.66 ± 49.30 | 226.00 ± 53.4 | 288.33 ± 52.71 | 467.33 ± 11.97*** | 216.00 ± 30.55* |
| ALT (U/L)       | 69.16 ± 9.31  | 63.16 ± 15.44 | 68.00 ± 16.10 | 100.16 ± 6.41*** | 61.00 ± 8.06* |

*p < 0.05 compared to control group (T−). **p < 0.01 compared to control group (T−). ***p < 0.001 compared to control group (T−).

Table 2 Effects of HP and the essential oil of Lavender on urea and creatinine levels in blood. The results are expressed as mean ± SEM, n = 6 mice/group.

| Parameters      | T−       | T+       | LOEO     | TOX      | LOEO+T   |
|-----------------|----------|----------|----------|----------|----------|
| Urea (mmol/L)   | 26.71 ± 6.76 | 22.033 ± 4.08 | 20.91 ± 8.50 | 12.31 ± 0.39** | 34.68 ± 9.54* |
| Creatinine (µmol/L) | 59.00 ± 18.05 | 49.5 ± 17.74 | 53.93 ± 16.76 | 12.21 ± 0.43*** | 50.26 ± 10.91* |

*p < 0.05 compared to control group (T−). **p < 0.01 compared to control group (T−). ***p < 0.001 compared to control group (T−).
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3.4.4 Histological examination

The cellular architecture of the Liver section of mice in control group (T−), positive control (T+) and in the group treated only with the essential oil of Lavender (LOEO) showed normal polyhedral cells, arranged in bays with central nuclei sometimes binuclear and granulated cytoplasm, separated one from another by sinusoidal capillaries with central vein and radiating cords of hepatocytes. However, in the HP-treated group, the histological section showed a remarkable damage in this organ. Furthermore, degeneration of hepatocytes, congestion of the central portal vein where there was an infiltration of leucocytes, dilatation of sinusoids and hemorrhage were clearly observed compared to control group (T−). The (LOEO + TOX) group preserved almost the same composition and the same cellular architecture (Fig. 4). The kidney section in the negative and positive control groups, as well as the LOEO-treated group, showed normal structure, normal glomeruli and tubulointerstitial cells. In contrast, the histological section of the kidney in the (TOX) group revealed a significant degeneration compared with the control mice. In fact, we observed tubular degeneration manifested by dilatation and congestion of the central portal vein. In the (LOEO + TOX) group, the histological section showed a normal structure similar to the negative control group.

Table 3 Mean effect (± SEM) of LOEO on HP-induced damage in the concentration of enzymatic antioxidants in liver and kidney tissues of mice. n = 6 mice/group.

|        | Liver |        |        |        |        |        |        |        |
|--------|-------|--------|--------|--------|--------|--------|--------|--------|
|        | T−    | T+     | LOEO   | TOX    | LOEO+T | T−     | T+     | LOEO   | TOX    | LOEO+T |
| SODα   | 0.58 ± 0.03 | 0.67 ± 0.02 | 0.62 ± 0.05 | 0.31 ± 0.09** | 0.56 ± 0.13* | 0.35 ± 0.007 | 0.34 ± 0.01 | 0.35 ± 0.04 | 0.07 ± 0.00** | 0.37 ± 0.02* |
| CATβ   | 0.08 ± 0.03 | 0.08 ± 0.04 | 0.08 ± 0.04 | 0.04 ± 0.02** | 0.08 ± 0.01* | 0.08 ± 0.05 | 0.09 ± 0.03 | 0.09 ± 0.04 | 0.03 ± 0.02*** | 0.08 ± 0.01* |
| GPxγ   | 0.30 ± 0.02 | 0.30 ± 0.03 | 0.30 ± 0.02 | 0.19 ± 0.08** | 0.28 ± 0.01* | 0.17 ± 0.01 | 0.18 ± 0.02 | 0.18 ± 0.01 | 0.02 ± 0.04** | 0.10 ± 0.01* |

Values having different symbols on a same line showed significant difference (p < 0.05) compared to control group: α: U mg−1 protein; β: μmoles/H2O2 consumed min−1 mg−1 of protein; γ: U mg−1 protein. *p < 0.05 compared to control group (T−). **p < 0.01 compared to control group (T−). ***p < 0.001 compared to control group (T−).

Fig. 4 Microscopic observations of mice liver sections (T−, T+, TOX, LOEO and LOEO + TOX: 100 μm). (T−) normal control groups showing normal parenchymal architecture, (T+) positive control group given green tea, group showing normal structure similar to the negative control group, (TOX) Hydrogen-peroxide-treated group showing diffuse central and peripheral necrosis and destruction of the lobular architecture, (LOEO) mice treated with the essential oil of Lavender and (LOEO + TOX) showing repairing of liver structure. 1: increased leukocyte infiltration and congestion of the central portal vein, 2: dilatation of sinusoid, 3: degenerations of hepatocytes.
tation of urinary space and a remarkable atrophy of the glomerule. The administration of our essential oil was able to prevent these damages in (LOEO + TOX) group (Fig. 5).

4 Discussion

The main purpose of this study was to investigate the ameliorative effect of *Lavandula officinalis* essential oil against HP-induced hepatotoxicity and nephrotoxicity in mice. It was evidenced that HP is one of the most used peroxo-compounds found in several fields such as water sterilization, food packaging, and various steps in food processing. But repeated use of this agent poses several health risks. Actually, our study demonstrated that the administration of HP at 774 mg/kg to mice induced significant changes in hepatic and nephritic functions. Under our experimental conditions, we have determined the antiradical activity and the reducing power of LOEO. Also, we have deduced its beneficial effects that can be attributed to free radical scavenging activity as well as its ability to reduce ferric iron by antioxidants present in the oil. We can notice the remarkable protective potentiality of the essential oil of Lavender compared to other plants, which is in agreement with findings reported in previous studies\[14\]. The DPPH and FRAP tests indicated that Lavender is a good antioxidative agent which is able to scavenge free radicals and to protect cells from severe damages induced by Reactive Oxygen Species (ROS) that are the major cause of many diseases. Indeed, many authors like AlKofahi and Atta\[15\] showed the effectiveness of lavender against several diseases compared to other plants like *Rosmarinus officinalis, Piper nigrum*, and *Oriandum sativum*. We further explored *in vivo* the effectiveness of LOEO in preventing liver and kidney damage. We observed an important difference at the level of the biochemical parameters both in liver and kidney in (TOX) group compared with the nontreated group (T-) indicating a remarkable damage in these two organs. Our results are similar to those reported by researchers in the field of INRS technical and medical services who have proved that HP causes, in mice, a degeneration of the hepatic tissues and renal tubular epithelial cells, necrosis as well as inflammation and irregularities in the structure of the gastric wall\[16\]. In fact, peroxide has been shown to enter the portal venous system after being absorbed through gastric mucosa\[17\]. This may be attributed

![Photomicrographs of mice kidney](image)

Fig. 5 Photomicrographs of mice kidney (T−, T+, TOX, LOEO and LOEO + TOX: 100 μm): (T−) normal control groups showing normal parenchymal architecture; (T+) positive control group, showing normal structure similar to (LOEO) group given essential oil of Lavender; (TOX) Hydrogen-peroxide-treated group showing degenerative changes, (LOEO + TOX) showing repairing of renal tissue.
1: Atrophy of the glomerular and dilatation of urinary space, 2: tubular degenerations.
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...to the rapid and complete absorption of HP whose absorption coefficient for biological membranes is comparable to that of water. Consequently, it passes easily into the cells and interacts with molecules leading to an extensive penetration of adjacent tissues. Glucose and transaminases are good markers with metabolic activity inside the cells; otherwise, transaminases are present in several tissues (liver, heart, kidneys, muscles), thus reflecting the activity and destruction of the cells of these organs. The increase in the transaminase levels in the blood confirms the presence of a cellular injury, most often in the liver. The main causes of this increase in blood are liver abnormalities that may be toxic or infectious hepatitis, cirrhosis, etc. This is also in agreement with the results reported by Tsinalis and Binet who demonstrated the formation of toxic compounds in organic peroxide-treated lake water, thereby causing a potential toxic effect on fish hepatocytes. In the same context, our results indicated that renal biomarkers significantly decreased. Indeed, creatinin is produced in a constant amount and freely filtered in the glomerulus; it has long been the best endogenous marker of glomerular filtration. Furthermore, urea is subjected to an important tubular reabsorption, depending essentially on the quantity of toxic-free water present in the nephron, its excretion is consequently irregular. This biomarker is the simplest way to estimate the renal function and a single blood sample yields easily interpretable results. From these results, we can conclude that the renal function is affected by HP, which was previously proved by Arnay et al. who demonstrated that the intravesical injection of HP in mice leads to long-lasting inflammatory and overactive bladder, and many authors have also proved the important role of peroxide in mediating injury in renal tissues. We also studied the impact of HP on the oxidative status of the experiments by analyzing the rates of SOD, GPx and CAT. Our results showed an increase of lipid peroxidation TBARS in the liver and kidney compared to control which was confirmed in recent studies showing that HP induced an oxidative stress in these two organs in adult mice by causing the formation of ROS and, consequently, significant cell damage. Indeed, the peroxidative damage causes lipid breakdown leading to a final product: malondialdehyde (MDA). The increased level of MDA can be explained by the formation of radicals that constitute the major cause of cellular membrane damage. The elevation of TBARS was accompanied by a decrease of the activities of antioxidative enzymes when compared to normal control mice. Under our experimental conditions, the decrease of SOD levels can be explained by the accumulation of highly reactive species that damage several constituents in the cell. This is the same case as that in a study by Shah who showed that HP induced an oxidative damage. Furthermore, levels of GPx and CAT decreased. Our study, therefore, showed an imbalance in the red-ox status in favor of prooxidants causing hepatic and renal injuries by oxidative stress. Simultaneously, pretreatment of HP-administered mice with LOEO enabled keeping the cells in homeostasis. Besides, our findings are confirmed by histopathological sections of the liver and kidney. In the control group, we had regular morphology. In contrast, remarkable and severe changes were noticed in the cytoarchitecture of these organs in the mice treated with HP compared to control, and we have proved the potential preventive effect of LOEO to combat these damages. Interestingly, herbal medicine, especially aromatherapy, has become of great importance as a rich source of biological compounds that have been shown to exert highly potent antioxidant properties. Also, it is worth noting that a phytochemical screening of the essential oil of *L. officinalis* leaves, which has been carried out in another study, revealed a complex composition with high levels of terpenic compounds with dominance of oxygenated monoterpenes and monoterpenic hydrocarbons. The identified major compounds were pentenone, propanal, methyl ethyl ketone, terpinen-4-ol, and isoborneol. These compounds attribute high pharmacologic activities to the essential oil of *L. officinalis*. Food technology is now based on the secondary metabolites of aromatic plants because they have important antioxidant powers and they can be good additives in the food industry. That is why the World Health Organization (WHO) considers this strategy in its important programs and suggests basic procedures to validate drugs in developing countries.

5 Conclusion

In conclusion, our results show that the performance of *Lavandula officinalis* essential oil makes it a promising candidate to resolve many metabolic and oxidative complications. This has been evidenced by its restoration of the normal hepatic and renal biochemical parameters interfering with the lipid peroxidation, adjustment of the enzymatic defense system, and prevention of severe cell damages that could be caused by reactive oxygen species.

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Competing Interests

The authors declare that they have no competing interests.

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