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Original Article

Antioxidant and Anti-Inflammatory properties of Essential Oils of Salvia officinalis on Immunopathological effects induced by Scorpion Envenomation

Propriétés Anti-oxydante et Anti-Inflammatoire de l'Huile Essentielle de Salvia officinalis sur les effets Immunopathologiques induits par l'Envenimation Scorpionique

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ABSTRACT

Introduction: Scorpion envenomation induces several complex clinical signs affecting various organs and systems at once, mainly the nervous and cardio-respiratory systems as well as systemic inflammatory response that can lead to death. Immunotherapy associated with symptomatic treatment remains the only means to combat this serious public health problem. The objective of this work is to explore the potential role of essential oils of *Salvia officinalis* on pathogenesis induced after scorpion envenomation. **Materials and Methods**: Animals were injected with a sublethal dose (0,5 mg/kg)of scorpion venom and treated with *Salvia* essential oils (0,5 %) (v/v). **Results:** Obtained results showed that the venom alone induced an inflammatory response in heart, lungs and livertissues characterized by an inflammatory cell infiltration (eosinophil and neutrophil) associated with hyperleukocytosis (60%). The scorpion venom induced also significant alterations in the histological architecture of these organs (heart, lungs and liver), anoxidative stress characterized by free radical overproduction (increase of NO concentration), a massive release of lipid peroxidation metabolites (MDA) and an inhibition of catalase activity with an increase in glutathione level. The use of *Salvia* essential oils allowed to an important reduction of immuno-inflammatory markers marked by significant decrease (80%) of eosinophil and neutrophil infiltration. Histological analysis confirmed the reduction of edema-forming and inflammatory cell recruitment in heart, liver and pulmonary parenchyma compared to envenomed mice. *Salvia* essential oils seemed to be more effective against oxidative stress (70 %) caused by scorpion envenomation. **Conclusion:** the Salvia species may represent natural, safe and effective treatment of scorpion envenomation. In recent decades, with the increase of pharmacological knowledge about the beneficial effects of sage especially *Salvia officinalis*, the herbal medicines with anti-oxidant, and anti-inflammatory effects have found to be effective in the development of novel natural drugs to prevent, control and treat health problems as well as more serious and complicated diseases.

KEY WORDS: Scorpion envenomation, pathological disorders, Essential Oils of *Salvia officinalis*, oxidative stress, inflammation

RESUME

Introduction: l'envenimation scorpionique induit des signes cliniques complexes affectant simultanément différents organes et systèmes, principalement les systèmes nerveux et cardiorespiratoire, ainsi qu'une réponse inflammatoire systémique pouvant entraîner la mort. L'immunothérapie associée au traitement symptomatique reste le seul moyen de lutter contre ce grave problème de santé publique. L'objectif de ce travail est d'explorer le rôle potentiel des huiles essentielles de *Salvia officinalis* dans la pathogenèse induite après envenimation scorpionique. **Matériels etMéthodes:** Les animaux ont reçu une dose sublétale (0,5 mg / kg) de venin de scorpion *Androctonus australis hector (Aah*) et ont été traités avec des huiles essentielles de *Salvia officinalis* (0,5 %) (v/v). **Résultats:** Les résultats obtenus ont montré que le venin seul induit une réponse inflammatoire au niveau du cœur, du poumon et du foie, caractérisée par une infiltration des cellules inflammatoires (eosinophiles et neutrophiles), associée à une hyperleucocytose (60%). Le venin de scorpion induit également des altérations significatives sur l'architecture tissulaire de ces trois organes, un stress oxydatif caractérisé par une surproduction de radicaux libres (augmentation de la concentration du NO), une libération massive de métabolites de la peroxydation lipidique (MDA) et une inhibition de l'activité de la catalase avec une augmentation du taux de glutathion. L'utilisation des huiles essentielles de *Salvia* a permis une réduction importante des marqueurs immuno-inflammatoires (80%) caractérisée parune diminution significative (80%) de l'infiltration des eosinophiles et neutrophiles. L'analyse histologique a confirmé la réduction de l'oedème formé et le recrutement des cellules au niveau du cœur, poumons et foie comparé avec les souris envenimées. Les huiles essentielles de Salvia semblent être très efficaces contre le stress oxydant (70%) induit par l'envenimation scorpionique. **Conclusion:** *Salvia officinalis* peut représenter un traitement naturel, sûr et efficace de l'envenimation scorpionique. Au cours des dernières décennies, avec l'avancement des connaissances pharmacologiques sur les effets bénéfiques de la sauge, en particulier de *Salvia officinalis*, les médicaments à base de plantes avec des effets anti-oxydants et anti-inflammatoires se sont révélés efficaces dans le développement de nouveaux médicaments naturels destinés à prévenir, contrôler et traiter les problèmes de santé graves et complexes.

Mots clés: Envenimation scorpionique, Huiles essentielles de *Salvia Officinalis*, stress oxydatif, inflammation

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1. Introduction

Scorpion envenomation is a real medical emergency and life hazardproblem in many countries; it is a potential cause of morbidity and mortality, especially among children. Neurotoxins are the most active components of thescorpion venom responsible for the toxic effects induced after scorpion envenomation. They induce amassive release of neurotransmitters during stimulation of sympathetic and parasympathetic of autonomic nervous system which can lead to cardio-respiratory failure and even to death. The pathophysiological disturbances caused by scorpion venoms are not exclusively assigned to the released neurotransmitters. The activation and the release of inflammatory mediators may also play a potential role in the induced pathogenesis. Several studies showed that the biological disorders following scorpionic envenomation are due to an inflammatory response characterized by the release of various inflammatory mediators, including cytokines, reactive oxygen species, and nitric oxide (NO) [1-3].

It has been also reported that scorpion venom can affect the immune system by mobilizing leukocytes and other inflammatory cells [4-7].

Previous studies have demonstrated that the activation of systemic inflammatory response induced by *Androctonusaustralis hector* (Aah) venom leads to a massive production of inflammatory mediators [5,8]. These inflammatory mediators are expressed in response to the toxins; they play an important role in the pathogenesis of envenomation and appear to have a deleterious effect on patients and on experimental animal exposed to the scorpion venom [8, 9].

The oxidant and antioxidant balance is an important determinant of immune cell function, not only for maintaining the integrity and functionality of membrane, cellular proteins, and nucleic acids but also for the control of signal transduction and gene expression in immune cells [9]. The inflammatory cells produce reactive oxygen species that react with

NO to form NO-derived inflammatory oxidants that damage tissues.

The anti-scorpion therapy is based on two approaches: i) a symptomatic treatment, adapted to the type of clinical symptoms observed and ii) the immunotherapy as a specific treatment. The use of the phytotherapy could be also associated.

Treatment of scorpion envenomation by plants remains right now unexplored. The validation of the usefulness of various species could form the basis for their use as alternative treatments.

Salvia officinalis L. (sage), is an aromatic plant belonging to the Lamiacea family. It grows spontaneously along the entire Mediterranean basin; it is quite common in Algeria.

S. officinalis is used for the consumption of fresh foods as a tasty flavoring food either in the form of dried leaves or essential oil, in herbal medicine and in the cosmetic industries [10].

Essential Oils of *Salvia officinalis* is known as the Functional novel Natural Medicine. Salvia Essential oils are considered important for drug development, as they are endowed of pharmacological activity and used in Asia, Middle East, China and India. Salvia has been used in traditional medicine against oxidative stress, free radical damages, angiogenesis, inflammation, bacterial and virus infection [11].

The present study was undertaken to explore the benefits of the essential oils of *Salvia officinalis* as a preventive and symptomatic treatment against scorpion envenomation.

2. Materials and methods

A. Materials

1. Biological Samples

1.1. Venom

Androctonusaustralis hector (Aah) venom was obtained from Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences of USTHB. It was lyophilized and stored at 4°C.

1.2. Essential oils

Essential oils of *Salvia officinalis*was obtained by hydrodistillation for 3h using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia.

1.3. Animals

NMRI mice (20 ± 2 g body weight) were obtained from the animal breeding facility of Faculty of Biological Sciences, USTHB. They were housed in controlled temperature and humidity rooms, and received food and water *ad libitum*, with a natural cycle of light and darkness. Animals were used according to the European Community rules of the EthicalCommittee for Animal Welfare. The experiments were achieved in line with the current guidelines for the care of laboratory animals.

2. Non biological Materials

The chemicals and reagents used were purchased from Sigma (St. Louis, USA) or Merck (Darmstadt, F.R.G) and were of analytical grade.

B. Methods

1. Essential oils of *Salvia officinalis* toxicity

Any therapeutically active substance is potentially toxic; everything will depend on thedaily dose and route of administration.

Ranges of different essential oil concentrations of sage, ranging from 0.1% to 10% (v/v), were tested to determine the optimal, safe and effective dose against the effects of a sublethal dose of Aah venom. The obtained results showed that the dose of 0.5% (v/v) is the optimaldose that justifies its use in this study.

2. Experimental Protocol of envenomation

Animals of experiments were divided into three groups (10 mice per group). The first group served as control was injected with saline solution (NaCl 0.9% by s.c. route); Group 2 received a sublethal dose of Aah venom (0.5 mg/kg body weight by s.c. route); Group 3 corresponds to the treated animals with Essential Oils of *Salvia officinalis.*

Animals were humanely sacrificed at 24 hours after envenomation. Blood was then collected and sera were obtained after centrifugation at 3000 *g* for 10 min and kept at 4°C until use. Organs and tissues (heart, lungs and liver) were homogenized in physiological saline solution. Homogenates were centrifuged at 4000 *g* for 20 min and supernatants were used as tissue extract for the various

experiments. Biomarkers of inflammatory response and oxidative stress were evaluated at 24 h after envenomation.

3. Anti-inflammatory effect of Essential Oils of *Salvia officinalis*

3.1. Peripheral blood cell counts

Blood samples were collected in EDTA tubes 24 h after Aah venom injection. A hemocytometer (ADVIA, Hematology system) was used for cell count.

3.2. Evaluation of inflammatory cell infiltration

Neutrophil accumulation and activation were estimated by evaluating myeloperoxidase (MPO) activity as previously described (Coelo et al., 2007). Samples were mixed with orthodianisidine (0.167 mg/ml) and H_2O_2 (0.4 mM) in phosphate buffer. Absorbance was readat 460 nm.

The extent of eosinophil accumulation in the biological samples was measured by assaying eosinophil peroxidase activity (EPO) as previously described [12]. Samples were mixed with Tris–HCl buffer containing OPD (10 mM) and H_2O_2 (0.4 mM). Absorbance was readat 490nm using an ELISA reader after incubation for 1h at room temperature in the dark.

4. Anti-oxidant effect of Essential Oils of *Salvia officinalis*

Pro-oxidant (malondialdehyde, nitrite) and antioxidant (catalase, glutathione) markers were measured in tissue homogenates (Heart, lungs and liver) and in sera.

4.1. Measurement of malondialdehyde (MDA)

The Measurement of MDA concentration is carried out using the thiobarbituric acid (TBA) at (100°C) in acid medium and in presence of SDS. The absorbances were determined at 532 nm. The results were expressed in nM/100 mg of tissue.

4.2. Measurement of nitric oxyde (NO)

Nitric oxide concentrations in serum and in brain, heart, liver, and lungs tissues were determined byassaying its breakdown products, nitrate and nitrite, using the Griess method. Aliquots of sample deproteinized [13]were incubated with equal volumes of Griessreagent. The absorbance was measuredby spectrophotometry at 543 nm.

4.3. Measurement of the glutathione

The determination of the reduced glutathione content (GSH) in homogenatetissues was carried out by using the 5,5 dithiobis 2 acid nitrobenzoic (DTNB). Its reduction by the glutathion leads to the compound formation that absorbs at 405 nm, 2-nitro-5-thiobenzoic acid (TNB). The concentration ofGSH was deduced from a molar extinction coefficient of13.6mM−1 cm−1. Results were expressed in mM per 100 mg of tissue.

4.4. Measurement of the catalase activity

The measurement of catalase activity is based on its capacity to transform H_2O_2 in phosphates buffer pH 7. The reduction in the absorbance was measured during 3 min at 240 nm. The enzymatic activity was expressed in UI/100 mg of tissues.

5. Histological analysis

Heart, lungs and liver collected from animals were immersed in formol fixative solution (10%) for 48 h. It was embedded in paraffin, sliced (7 µm) and stained with hematoxylin and eosin for microscopic examination (Motic Digital Microscope PAL System).

6. Statistical analysis

All results were expressed as the mean ± SD. The statistical significance of differences between groups was analyzed by a Student *t*-test. Data were considered statistically significant if p-values were <0.05.

3. Results

1. Evaluation of anti-inflammatory effect of Essential Oils of *Salvia officinalis*

The injection of scorpion venom by subcutaneous route to animals induces an important inflammation response characterized by hyperleukocytosis marked

by a significant increase of leukocytes and lymphocytes associated with an important decrease of monocytes, neutrophils and eosinophils in the peripheral blood of injected mice with Aah venom compared to the controls(NaCl) (**Figure 1**).

Evaluation of MPO and EPO activities, markers of inflammation is used as an index of activation and infiltration of neutrophils and eosinophils into the inflammatory sites. The MPO and EPO activities in tissue homogenates of injected mice with Aah venom were significantly higher compared to that of controls (NaCl) (**Figure 2**).

The treatment of animals with the essential oils of sagelimits the leukocytosis caused by Aah venom. Indeed, the use of essential oils after envenomation normalizes the rate of total leucocytes but also the levels of studied cell populations (lymphocytes, monocytes, neutrophils, eosinophils, and basophils).

Previous study showed a significant reduction in total leukocytes following the use of a *Salvia officinalis* in rats with turpentine oil-induced inflammation.
Similarly, reduction of enzymatic activities of meyloperoxidase and eosinophil peroxidase has been observed in treated animals with essential oils of *Salvia officinalis*.

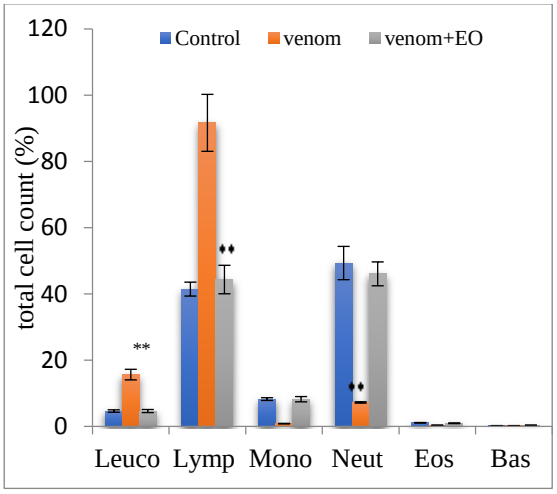
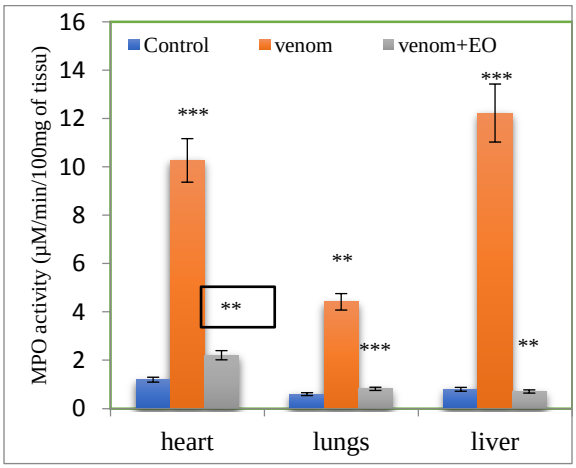


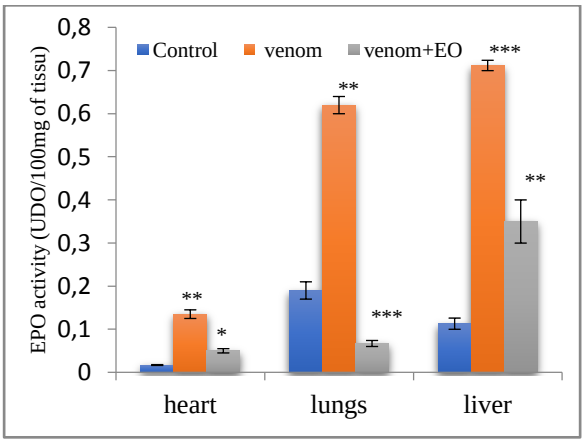
Figure 1: Leukocyte count in the peripheral blood 24 h after injection of venom (0.5 mg/kg by s.c. route) in the presence and absence of treatment with Essential Oils of *Salvia officinalis*
Values represent the means ± S.D. (n = 3). *p ≤ 0.05; **p ≤ 0.01

2. Evaluation of anti-oxidant effect of Essential Oils of *Salvia officinalis*

The study of the effects of Essential Oils of *Salvia officinalis* after scorpion envenomation on oxidative balance was carried out by the cellular pro- and antioxidant balanceanalysis (**Figures 3 and 4**). This study was realized after envenomation of mice by subcutaneous injection of sublethal dose of Aah venom (0.5 mg/kg) and treatment by Essential Oils of *Salvia officinalis* (0.5 %).



(A)



(B)

Figure 2: Determination of myeloperoxidase MPO (A) and eosinophil peroxidase EPO (B) activities, 24 h after injection of venom (0.5 mg/kg by s.c. route) in the presence and absence of treatment with Essential Oils of *Salvia officinalis*.
Values represent the means ± S.D. (n= 3). *p ≤ 0.05; **p ≤ 0.01;***p ≤ 0.001

2.1. Effect of Essential Oils of *Salvia officinalis* on lipidic peroxidation

In order to evaluate the extent of the lipidic peroxidation caused by Aah venom on various tissues, a specie reactivate of the thiobarbituric, malondialdehyde acid (MDA), was measured in heart, lung and liver tissues of envenomed mice by a sublethal dose of venom injected by subcutaneous route.

Obtained results showed an increase in the MDA levels in heart, lung and liver homogenates of envenomed mice compared to that of animal controls (**Figure 3 A**).

Salvia officinalis appeared to significantly reduces MDA levels [14]. Obtained results showed that the use of Essential Oils of *Salvia officinalis* in the treatment of envenomed animals by Aah venom is able to reduce the MDA level in the studied organs.

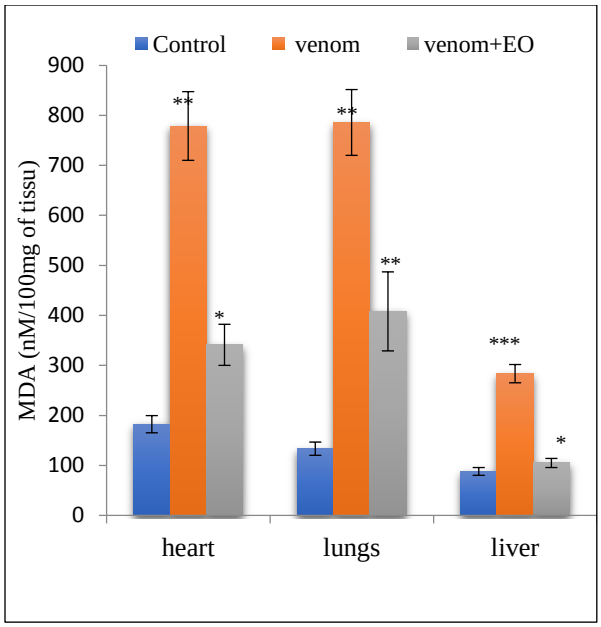
2.2. Effect of Essential Oils of *Salvia officinalis* on nitric oxide release

The nitric oxide (NO) is free radical synthesized after L-arginine conversion into NO, by NO synthases (NOS). It plays animportant role in severalphysiological and physiopathological processes, as it is one of the oxidative stress markers.

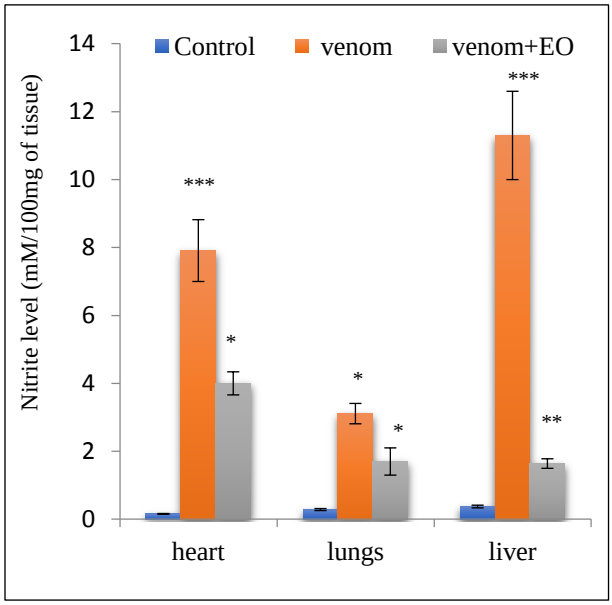
The obtained results showed a significant increase in NO levels in tissue supernatants of mice after 24 hours following the envenomation compared to the control (**Figure 3B**). However, the treatment of mice with essential oil decreased the NO level in the tissue compartments compared to those of envenomed animals. These results could be explained by the antioxidant effect of one of the phenolic constituents of the essential oils of *Salvia* [15].

2.3. Effect of Essential Oils of *Salvia officinalis* on catalase activity

The catalase is an antioxidant enzyme present in various tissues; it is responsible for the hydrogen peroxide conversion into water and oxygen [16]. The evaluation of the catalase activity in mice after 24 hours of envenomation showed a reduction in this activity compared to the control (**Figure 4A**).



(A)



(B)

Figure 3: Evaluation of oxidative stress biomarkers: MDA (A) and Nitrite (B), 24 h after injection of venom (0.5 mg/kg by s.c. route) in the presence and absence of treatment with Essential Oils of *Salvia officinalis*

Values represent the means ± S.D. (n= 3)

*p≤0.05; **p ≤ 0.01; ***p ≤ 0.001

The decrease of catalase activity could be explained by an excessive production of hydrogen peroxide during the oxidative stress induced by *Aah* venom. Indeed, in the presence of high levels of H_2O_2 the catalase activity was inhibited [16]. Other studies reported an important increase in hydrogen peroxide release by the macrophages stimulated *in vitro* with the *Tityusserrulatus*or *Androctonusaustralis hector* venoms [17-19].

The treatment of animals with the essential oils of *Salvia officinalis* causes a significant increase in catalase activity. The increase of catalase activity was observed after treatment of animals with *Salvia officinalis* tea for 14 days [20].

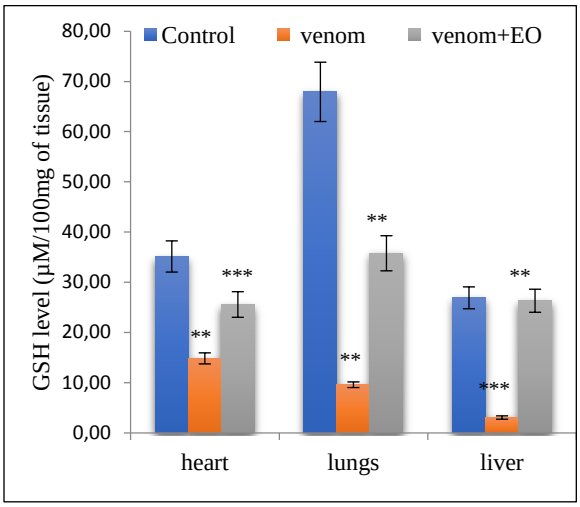
3. Effect of Essential Oils of *Salvia officinalis* on glutathione levels

The non-enzymatic antioxidant balance evaluated by the measurement of glutathion (GSH) in the tissue supernatants of envenomed mice with Aah venom showed a decrease in GSH levels compared to the control. The treatment of animals with the essential oils of *Salvia officinalis* seems to improve the cellular antioxidant potential by increasing the rate of GSH in the studied organs compared to the envenomed animals (**Figure 4B**).

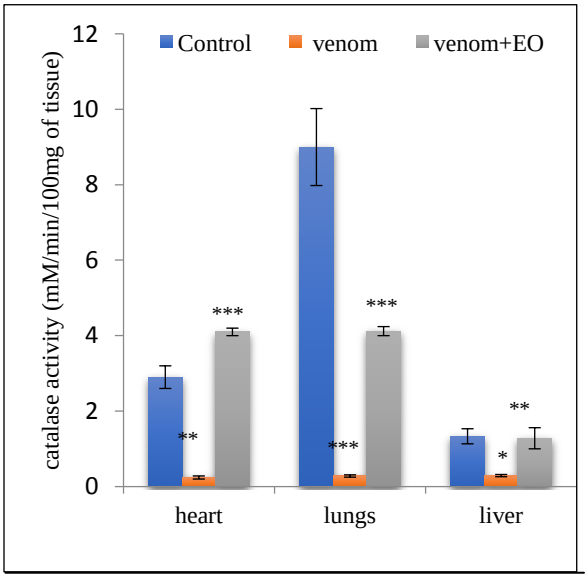
4. Effects of Aah venom on heart, lung and hepatic tissues

Histological analysis revealed severe structural alterations in myocardium, pulmonary and hepatic tissues of injected animals with sublethal doses of Aah venom. These alterations were characterized by: i) a disorganization of hepatic tissue architecture with dilated portal vein, irregular hepatocytes, hemorrhagic edema, congestion, hemorrhage and cell infiltration; ii) alteration of myocardic tissue characterized by hemorrhage, interstitial edema, necrosis of myocardiocytes and fibber hypertrophy associated with inflammatory cell infiltration; iii) pulmonary tissue damage characterized by hemorrhage, interstitial edema and thickening of interalveolar septa. Thickened and altered alveolar walls exhibited abnormal accumulation of inflammatory cells (**Figure 5**).

Administration of *Salvia officinalis* essential oilsafter Aah venom injection seems to restore the hepatic, cardiac and pulmonary tissue damage induced by venom and to reduce the leukocyte infiltration observed in tissues of envenomed animals.



(A)



(B)

Figure 4: Evaluation of oxidative stress biomarkers: glutathione GSH (a) and catalase CAT (b); 24 h after injection of venom (0.5 mg/kg by s.c. route) in the presence and absence of treatment with Essential Oils of *Salvia officinalis*. Values represent the means ± S.D. (n= 3).*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

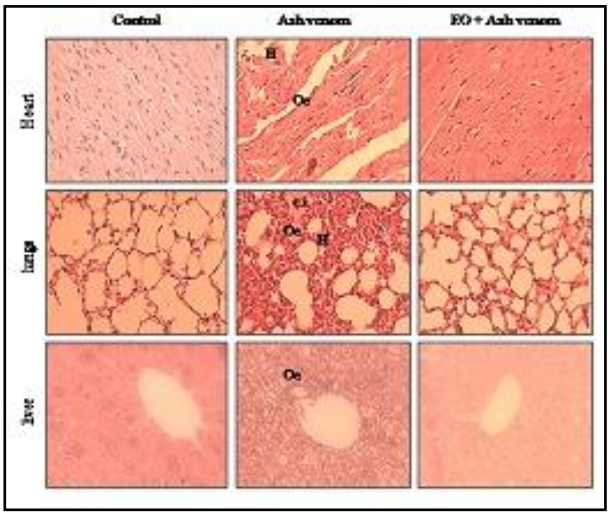


Figure 5: Histology analysis of myocardium, pulmonary and hepatic tissues of animal controls, envenomed animals with *Aah* venom and envenomed animals with *Aah* venom and treated with *Salvia officinalis* essential oil (EO)
H: hemorrhage, Oe : edema, c.i. : cell infiltration.
Coloration Hematoxylin-eosin. X400

4. Discussion

Scorpionic envenomation is a real threat in many countries of the world, in particular in Northern Africa, Central and Southern America, the Middle East and India. Anti-venom therapy is based on two approaches: symptomatic and specific treatment (immunotherapy). In this study, a phytotherapeutic approach using *Salvia officinalis* as a symptomatic treatment was evaluated against scorpion envenomation based on its beneficial anti-inflammatory and anti-oxidant properties. The essential oil of *Salvia officinalis*was used as pharmacologically active molecules after experimental scorpionic envenomation. The obtained results showed that envenomed animals with a sublethal dose of Aah venom causes a significant inflammatory response characterized by serum hyperleukocytosis associated with intense activation and infiltration of inflammatory cells in tissue.

The migration of leukocytes from the vascular system to the injured area is a key event in inflammation [8]. Previous studies reported that the scorpion envenoming induces activation of the inflammatory cascade and release of immunological mediators responsible for leukocyte recruitment [5, 9].

Aah venom induces also an installation of oxidative stress characterized by an overproduction of free radicals (NO), a massive release of a lipid peroxidation metabolite (MDA), an inhibition of catalase activity associated with basal glutathione levels.

The increase of NO level was also reported after experimental envenomation by *Androctonusaustralis hector*, *Tityuszulianus*and of *T. serrulatus* venoms [21].The overproduction of free radicals (NO) could be explained by the activation of NO synthase after neurotoxin binding on their cellular targets.

The increase of MDA levels indicates the alteration of cell membrane by the membrane lipid oxidation. This disorder could be due to the action of reactive species of oxygen and NO, which are released during the oxidative process after scorpion envenomation.

These species (ROS) can interact and exert their toxic effects by causing the lipid peroxidation which is the

cause of the alteration of the integrity of cellular membranes [22, 23].

A disturbance of antioxidant system is observed. It is characterized by a production of glutathion (GSH) and by a decrease of the catalase activity, suggesting their involvement in the molecular mechanisms of cellular detoxification in response to the venomcomponents.

The use of essential oils of *Salvia officinalis* after scorpionic envenomation showed almost total protection of animals against the deleterious effects caused by Aah venom. Indeed, oral administration of these oils has reduced the inflammatory response and oxidative stress in all tissue compartments of envenomed animals. It was reported that *Salvia officinalis* essential oils have a significant inhibitory effect on the migration of inflammatory cells to peri-vascular tissues by reducing the expression of adhesion molecules, the synthesis of inflammatory mediators and the release of cytokines [24]. Similarly, *Salvia officinalis* essential oils, mainly its carnosic acid and carnosol components, are able to inhibit significantly the migration of neutrophils and eosinophils to inflammatory sites [25, 26].

A reduction of oxidative stress was also observed, characterized by an improvement of the enzymatic and non-enzymatic antioxidant capacity by reactivating the catalase activity and increasing the cellular GSH levels. Similarly, basal NO and MDA levels were also observed. *Salvia officinalis* reduces the level of malondialdehyde probably due to the decrease of OH radicals [20]. This plant characterized by a high level of flavonoids, which

could have a role in the reduced lipid peroxidation by decreasing superoxide radicals resulting from stress in rats [27].

The protective effect of essential oils may be due to their content of phenolic compounds such as thujone, cineole and camphor that can be purified and useful for repairing tissue damage induced by oxidative stress [28].

The treatment of envenomed animals with *Salvia officinalis* essential oil seems to have an interesting effect in the reduction of inflammatory and oxidative responses induced by scorpion experimental envenomation. These results are likely to be exploited in a phytotherapy-based anti-envenoming treatment.

Conclusion

Androctonus australis hector scorpion venom is able to increase the sympathetic nervous system activity and provoke a systemic inflammatory response characterized mainly by an increase of leukocyte cell count in the peripheral blood and their recruitment in site of injury, associated with tissue damage and oxidative stress. The pathophysiology of these events is complex; it seems to be mediated by the massive release of inflammatory mediators and reactive oxygen species. The use of essential oils of *Salvia officinalis* could be beneficial to improve the treatment in the future mainly in severely envenomed patients by scorpion venom.

Conflicts of interest

The authors declare that they have no conflict of interest

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