Anti-inflammatory effect of *Helix aspersa* extracts in sephadex-induced model of lung inflammation: possible modulation of neutrophils

Mebirouk Romeila¹,², Naimi Dalila*²,³

¹Faculty of Medicine, Salah Boubnider University, Constantine 3, Ali Mendjeli University Center 25100 Constantine, Algeria
²Laboratory of Bioengineering, Ecole Nationale Supérieure de Biotechnologie, Taoufik Khaznadar of Constantine, Algeria
³National Higher School of Biotechnology, Taoufik Khaznadar, Constantine, New University Center Ali Mendjeli, BP E66, 25100 Constantine, Algeria

Article History:

Received on: 12 Aug 2021
Revised on: 10 Sep 2021
Accepted on: 14 Sep 2021

Keywords:

anti-inflammatory effect, *Helix aspersa* extracts, histopathology, lung inflammation, myeloperoxidase

The focus of our study is to compare the anti-inflammatory effect of extracts prepared from Algerian mollusk called *Helix aspersa*, on induced lung inflammation in rats: aqueous AE, hydroalcoholic HE and organic OE extracts. Animals were classified into six groups: control group T was instilled with NaCl (0.9%) in the trachea, negative control group was instilled with sephadex in trachea (5mg/mL/Kg), positive control group was instilled with sephadex and treated with an anti-inflammatory drug, groups SAE, SHE and SOE were instilled with sephadex and treated intraperitoneally with aqueous, hydroalcoholic and organic extracts respectively (4mg/mL/Kg). 24h after instillation, we sacrificed animals, blood samples and bronchoalveolar fluid lavage (BALF) were collected. Lungs were preleved for histological investigation. We measured myeloperoxidase activity (MPO) in BALF. Results showed sephadex induces a massive granuloma in lungs. HE and OE decreased leukocyte count in BALF and blood. AE increased leukocyte count in blood and decreased it in BALF. The three extracts decreased MPO activity in BALF, reduced number of inflammatory cells in the alveoli and protected lung from sephadex beads injuries. Both AE and HE reduced edema in lung. We conclude that treatment with *Helix aspersa* had an anti-inflammatory effect on lungs inflammation.

*Corresponding Author
Name: Naimi Dalila
Phone:
Email: D.Naimi@ensbiotech.edu.dz

ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v12i4.4904](https://doi.org/10.26452/ijrps.v12i4.4904)

INTRODUCTION

Inflammation is a natural protective response of the immune system to remove an injurious stimulus. It is a complex process involving a multifactorial network of chemical signals. After activation, inflammatory cells release many inflammatory mediators, these include cytokines, chemokines, proteases, histamine, myeloperoxidase, and reactive species of oxygen. They induce blood vessel dilatation, recruitment of other inflammatory cells, realize of other inflammatory molecules. However, inflammation can persist longer than necessary and evolve towards chronicity, causing severe pathologies characterized by significant histopathologic changes and tissue destruction. Excessive production of pro-inflammatory mediators may be the cause ([Mantovani et al., 2008](https://doi.org/10.26452/ijrps.v12i4.4904)).
tory response in the lung is characterized by the influx of circulating inflammatory cells into the tissue, including eosinophils and neutrophils. These inflammatory cells could damage the lung tissue via the production of pro-inflammatory cytokines, proteases, myeloperoxidase, lysozymes, and reactive oxygen species, which leads to the formation of edema. The MPO is an enzyme contained in neutrophils of lung parenchyma and BALF. It has been described as a pro-inflammatory enzyme that is most abundantly expressed in neutrophil granules. It is a peroxidase enzyme that plays a critical role in the development of lung inflammation. Its enzymatic activity contributes to neutrophil extravasation (Serteyn et al., 2003).

The common anti-inflammatory drugs are non-steroidal anti-inflammatory drugs that target cyclooxygenase (COX), cytokines (TNF–α, IL-1 and IL-6) and the NF-κB transcription factor. The use of anti-inflammatory drugs is facing many side effects such as gastrointestinal diseases, immunodepression and autoimmune diseases (Helms, 2006; Rang and Dale, 2012).

Previous studies have reported biological effects in vitro and in vivo of several extracts of the terrestrial gastropod Helix aspersa, including antitumor activity (Ouar et al., 2013; Mebirouk et al., 2016) and protective effect against induced colitis and antioxidant activity (Hatuikulipi et al., 2016).

In order to evaluate the anti-inflammatory effects of the aqueous, hydroalcoholic and organic extracts prepared from the foot of the snail Helix aspersa, a model of pulmonary inflammation was developed in female rats by the intra-tracheal instillation of Sephadex G-200.

MATERIALS AND METHODS

Biological materials

Helix aspersa

Helix aspersa was collected in Constantine-ALGERIA, in spring season after the animal’s winter sleep; it was maintained in plastic backs in the laboratory at room temperature (25±1°C). Snails were fed lettuce, carrot and cucumber and had access to water.

Wistar Albinos rats

Thirty Wistar albino rats (body weight between 80-120g) were used in this study. These animals came from the animal house of Pasteur institute – Algiers, ALGERIA. They were acclimatized one month before the experience to experimental conditions in laboratory cages. They had free access to drinking water and traditional food throughout the experiments at room temperature (25±2°C), 12h light/dark cycle and humidity 50-60%. The research was conducted according to the guidelines of the Institutional Animal Ethics Committee (IAEC).

Chemical material

1. Sephadex G-200 (Sephadex G-200 Superfine, Pharmacia). It consists of a crosslinked dextran polymer.

2. Nonsteroidal anti-inflammatory drug (Ibuprofen, 200mg, MERINAL), NSAID works by reducing molecules that cause inflammation in the body.

Preparation of Helix aspersa extracts

Aqueous, hydroalcoholic and organic extracts

Preparation of extracts was performed according to protocol elaborated by Mebirouk et al. (2016) with some modifications. Briefly, Helix aspersa foot homogenate was lyophilized, extracted three times with distilled water and filtered on Büchner. The filtrates obtained were lyophilized and constituted the aqueous extract (AE). The resulting pellets were extracted once with ethanol (80%) and twice with absolute ethanol and then filtered on Büchner. Alcohol was evaporated from the resulting filtrates with a rotary evaporator (at 40°C). The obtained extract was adjusted with distilled water and then extracted three times with dichloromethane in a separatory funnel. Two phases with different densities were obtained, aqueous and organic phases. Aqueous phase constitutes the hydroalcoholic extract (HE), it was lyophilized. Dichloromethane was removed from the organic phase with a rotary evaporator (at 40°C) and the resulting extract constitutes the organic extract (OE).

The protocol preparation details are summarized in the Figure 1, the three extracts were prepared from Helix aspersa fresh foot homogenate using distilled water, ethanol and dichloromethane as solvents; then, the extracts were lyophilized and diluted. After many tests, we diluted AE in culture medium RPMI (1640) and both HE and OE in NaCl (0.9%).

Hemagglutinating activity of aqueous extracts and hydroalcoholic extracts

The hemagglutinating activity was performed on the microtiter plate. Briefly, we add 50μL of the erythrocyte suspension diluted with saline solution (4%) to 100 μL of each extract (AE and HE). The microplate is stirred and incubated for 45 min at 37 °C. Observation of agglutination is visible to the naked eye.
Induction of lung inflammation

Acute lung inflammation was performed with Sephadex G-200 in rats, according to (Evaldsson et al., 2011) protocol, with some modifications. Thirty Wistar albinos rats were classified into six equal groups (n=5 per group). Acute lung inflammation was induced by intra-tracheal instillation of 1 mL of a Sephadex solution (5 mg/mL/Kg), dissolved 12-24 hours before installing in a sterile physiological solution (0.9 %). The treatment of different groups is as follows,

1. Group T (control) received 1mL of a sterile solution of NaCl 0.9% by intra-tracheal instillation performed with a sterile syringe.
2. Group Sx (sephadex alone) received 1mL of the Sephadex solution (5 mg/mL/Kg) alone in the trachea.
3. Group Sibu (sephadex + Ibuprofen) received the Sephadex solution by intra-tracheal instillation and a dose of 30 mg/Kg body weight of Ibuprofen.
4. Group SAE received 1mL of sephadex solution by intra-tracheal instillation and 1 mL of the aqueous extract (1 mg/mL) intra-peritoneally,
thirty minutes after the instillation Sephadex.

5. Group SHE received by intra-tracheal instillation of 1mL Sephadex solution and 1 mL of the hydroalcoholic extract (1 mg/mL) intra-peritoneally, thirty minutes after the instillation of the Sephadex.

6. Group SOE received by intra-tracheal instillation of 1mL sephadex solution and 1 ml of the organic extract (1 mg/mL) intra-peritoneally, thirty minutes after the instillation Sephadex.

Animals were fasting overnight, blood samples were collected from the cavernous sinus 24 hours after the instillation, for counting total leukocytes under anaesthesia through the intraperitoneal injection of 5% chloral hydrate. A pulmonary lavage was performed with 1 mL of a sterile physiological solution injected into the trachea to collect the bronchoalveolar lavage fluid (BALF). The liquid is recovered in Eppendorf tubes and centrifuged at 400 g for 5 min at 4 °C. The supernatants were frozen at -30 °C until use. The cell pellets were diluted for counting white blood cells. After pulmonary washing, rats were sacrificed and the lungs were removed, weighed and fixed in formalin (4 %) for histological sections. The histological sections were stained with Hematoxylin-Eosin following the standard method and then observed by an OPTECH photo-microscope coupled with a camera (Canon-DS126181).

**Myeloperoxidase assay (MPO) in BALF**

MPO is carried out in the BALF according to the method of Bradley et al. (1982), with some modifications: we mixed 100 μL of BALF with 100 μL of potassium phosphate buffer (50 mM, pH 6) containing 0.5 % of HTAB (Hexadecyltrimethylammonium bromide from Sigma Life Science). The mixture obtained is frozen-thawed in liquid nitrogen and then ground with ultrasonic vibrations for 3 cycles using a Vibra Cell™ 75043 (Bio block Scientific, 750W max power). The homogenate obtained is centrifuged for 15 min at 40000 g at 4 °C. 100 μL of supernatant are mixed with 2.9 mL of phosphate buffer (50 mM, pH 6), which contains: 0.167 mg/mL of O-dianisidine dihydrochloride (Sigma-Aldrich) and 0.0005 % of hydrogen peroxide (H₂O₂). The absorbance is measured at λ = 460 nm.

**Determination of total proteins in BALF**

The total protein level in BALF (in supernatants) was measured according to the Bradford method (Bradford, 1976).

**Statistical analysis**

The values are expressed as mean±deviation (SD). The statistical signification of differences between the groups was analyzed by ANOVA. In all cases P<0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Hemagglutinating activity**

[Figure 2: Hemagglutinating activity in aqueous and hydroalcoholic extracts]

The hemagglutination activity assay revealed after 45 min of incubation with erythrocytes that AE showed no activity of erythrocytes used in the test. From (Figure 2), a: erythrocytes suspension added to the hydroalcoholic extract, b: erythrocytes suspension added to aqueous extract; the agglutination was observed just for the hydroalcoholic extract (a).

[Figure 3: Effects of *Helix aspersa* extracts on total leukocytes count (A): in blood, (B) in BALF]

**Total leukocyte count**

Cell counts of total leukocytes showed a very significant increase (p=0.005) in the Sx group (6.8x10⁹±0.95) cell/mm³, in the SAE group (7.8x10⁹±2.86) cell/mm³, and the SBu group (8.85x10⁹±2.5) cell/mm³ compared to the control group (5.58x10⁹±1.5) cell/mm³. However, we noted a significant decrease in leukocyte count in the SHE group (3.5x10⁹±1.64) cell/mm³. In the SOE group, we noted approximately the same leukocyte count (6.022x10⁹±0.94) cell/mm³ compared to the NaCl group (5.58x10⁹±1.5) cell/mm³. From (Figure 3A), We noted an increase in blood total leukocyte count in the group treated with aqueous extract. The opposite effect was recorded with the other extracts.

**Leukocyte count in BALF**
Cell count of leukocytes in BALF increased very significantly \((p=0.005)\) after sephadex beads administration \((294.4\pm384.2)\) cell/mm\(^3\) and a very significant increase in white blood cells in BALF \((p=0.00)\) in groups treated with NSAID \((1336\pm353.94)\) cell/mm\(^3\) comparing with non treated group \((944\pm324.46)\) cell/mm\(^3\). We also noted a very significant reduction in total leukocyte count after treatment with aqueous extract \((712\pm186.33)\) cell/mm\(^3\), with hydroalcoholic extract \((420\pm71.06)\) cell/mm\(^3\) and organic extract \((424\pm104.3)\) cell/mm\(^3\). From (Figure 3 B), In BALF, the three extracts decreased the leukocyte count.

**activation in BALF**

Activation of neutrophils in BALF was assessed by evaluating the MPO level. The results demonstrated a non-significant increase in MPO activity in BALF in the Sx group \((24.47\pm4.82)\) unit/mol of degraded \(\text{H}_2\text{O}_2\) compared to the NaCl group \((17.96\pm2.17)\) unit/mol of degraded \(\text{H}_2\text{O}_2\). We also found a non significant increase in the groups treated with the anti-inflammatory drug after instillation of sephadex \((23.79\pm9.32)\) unit/mol of degraded \(\text{H}_2\text{O}_2\) and in the group treated with SAE \((21.72\pm2.92)\) unit/mol of degraded \(\text{H}_2\text{O}_2\) compared to control group. MPO activity level in the SHE group was \((17.69\pm8.93)\) unit/mol of degraded \(\text{H}_2\text{O}_2\) and \((19.02\pm4.94)\) unit/mol of degraded \(\text{H}_2\text{O}_2\) in the SOE group. From (Figure 5), Data are shown as means±SD (n=5). Neutrophil activity was decreased after treatment with *Helix aspersa* extracts.

**The protein level in BALF**

After sephadex instillation, total protein concentration in BALF increased very significantly \((p=0.00)\) in Sx group \((1.59\pm0.22)\) mg/mL compared with control group \((1.28\pm0.15)\) mg/mL. Nevertheless, a very significant reduction \((p=0.00)\) was noted in rats treated with NSAID \((0.72\pm0.24)\) mg/mL and treated with *Helix aspersa* hydroalcoholic extract \((1.18\pm0.23)\) mg/mL. In SAE and SOE, we noted an increase in protein level \((1.47\pm0.12)\) mg/mL and \((1.37\pm0.22)\) mg/mL, respectively, compared to the control group. From (Figure 4), Data are shown as means±SD (n=5). ***P<0.001 compared with the control group). A decrease in protein concentration with the three extracts was noted.

**Effect of *Helix aspersa* extracts on neutrophil**

Effect of *Helix aspersa* extracts on neutrophil activity in BALF

Lungs are weighted after the experience to see if edema was decreased after treatment with *Helix aspersa* extracts. Indeed, in Sx, Sibu and SOE groups, we noted an increase in lung weight \((p>0.05)\): \((1.03\pm0.27)\) g, \((1.06\pm0.27)\) g, and \((1.01\pm0.34)\) g respectively compared to NaCl group \((0.81\pm0.13)\) g. The fluid influx corresponded to 31% in group Sx, 27% in Sibu and 25% in the SEO group in lung weight. No changes were noted in lung weight in SAE \((0.86\pm0.07)\) g and SHE \((0.81\pm0.13)\) g compared to the NaCl group. From (Figure 6), Data are shown as means±SD (n=5). Aqueous and hydroalcoholic extracts had a negative effect on lung weight. The organic extract did not affect this parameter.

**Histological findings**

To understand the cause of the increase in lung weight in some groups treated with *Helix aspersa* and their effect on lung tissue, histological sections were performed. Microscopic examination of the NaCl group showed normal pulmonary architecture, bronchiole with normal epithelium surrounded by a smooth muscle bundle. Macrophages, pneumo-

![Figure 4: Effect of *Helix aspersa* extracts on protein level in BALF](image)

![Figure 5: Effect of *Helix aspersa* extracts on MPO neutrophil activity in BALF](image)

![Figure 6: Lung weight changes after treatment with *Helix aspersa* extracts](image)
cytes type I and type II were identified in alveoli. From (Figure 7), Normal pulmonary tissue in the rats received NaCl 0.9% (A). Lung inflammation in the rats received Sephadex (B). Relieved lung inflammation in the rats treated with sephadex and AINS (C), sephadex and EA (D), sephadex and EH (E) and sephadex and EO (F).

However, microscopic examination of the group that received intratracheal instillation of sephadex showed a lung with an altered structure. The epithelial wall was degraded, and we noticed the presence of a granuloma close to the bronchiole. It consisted predominantly of macrophages and monocytes. The alveolar space was infiltrated by leukocytes that also contain sephadex beads. In animals treated with the anti-inflammatory, we noticed the presence of mucus in the bronchiole lumen with some inflammatory cells, and the lung parenchyma was altered.

In contrast, we noticed a low number of inflammatory cells in the alveoli. In groups treated with the aqueous extract and hydroalcoholic extract, we noticed a low number of inflammatory cells in the alveolar space and the respiratory epithelium was intact. Regarding the last group treated with the organic extract, we observed a normal respiratory epithelium. However, we found some inflammatory cells and lymphocytes in the vicinity of the bronchioles. We also identified epithelial cells, eosinophils and giant cells.

**DISCUSSION**

*Helix aspersa* is an edible terrestrial snail found in several countries, especially in Mediterranean countries and that has been used in therapeutic medicine since antiquity. To evaluate its anti-inflammatory effect, we prepared three types of extracts from the foot: the aqueous, hydroalcoholic and organic extracts. We used a model of pulmonary inflammation developed in rats by intra-tracheal instillation of Sephadex G-200. It is a model of acute inflammation of the bronchi and alveoli, and it is an ideal model for assessing the anti-inflammatory effects of biomolecules. Sephadex particles cross the bronchial epithelium and induce the chemotaxis of...
eosinophils. These cells cause the increase of the permeability of the vessels, the formation of edema, the contraction of the smooth muscle and the hypersecretion of the mucus by the goblet cells. The presence of eosinophils is the result of the production of IL-5. This cytokine induces the recruitment of other inflammatory cells into the lung that may be associated with increased expression of chemokines, cytokines, lipid mediators (Haddad et al., 2002). Granuloma induced by sephadex beads is composed of macrophages, monocytes, eosinophils? and multinucleated giant cells. The macrophage secretes proteases, leukotrienes? and chemotactic factors for neutrophils. In the lung tissue, neutrophils play a very important role in inflammation. These inflammatory cells accumulate and produce in the lung parenchyma granules containing enzymes such as elastase and myeloperoxidase. The action of these enzymes in the pulmonary parenchyma leads to the massive degradation of the elastic fibers of the bronchioalveolar wall. Elastic fibers are an important component of the bronchi and the alveolar wall; their destruction would cause the loss of elasticity and the formation of emphysema (Kierszenbaum, 2002).

Our study showed a very significant increase in leukocytes in BALF of the Sx group indicates lung inflammation. The observation of inflammatory cells in lung tissue, such as neutrophils, eosinophils and macrophages, explain the increase in the number of total leukocytes in blood and BALF. Several studies have shown the increase of inflammatory cells, principally eosinophils and neutrophils, in rats instilled with Sephadex in comparison with the control rats. Co-administration of Sephadex with the nonsteroidal anti-inflammatory drug (Sibu lot) did not decrease the level of total leukocytes in the blood. However, it decreased it in the BALF. This decrease could be due to its local effect in lung tissue by inhibiting inflammatory molecules that attract inflammatory cells. Treatment with the three aqueous, hydroalcoholic and organic extracts significantly decreased the total leukocyte count in BALF. This indicates a modulating effect of Helix aspersa extracts by inhibiting the chemotaxis of inflammatory cells in lung tissue. We also measured protein level in BALF to confirm that, and we found an increase of protein level in BALF of group Sx. It could indicate an increase in inflammatory cytokine production, chemokines, and lipid mediators. Previous studies have shown the activation of TNFα, leukotriene C4, D4 and E and prostaglandin E2 production following instillation of Sephadex in rat trachea (Zhipeng et al., 2012; Nagar et al., 2015). Treatment with NSAID induced a decrease in the concentration of proteins which confirms their anti-inflammatory effect by blocking the synthesis and production of some inflammatory mediators (lipid mediators) by acting on the COX pathway, including COX2 (Helms, 2006; Rang and Dale, 2012). The same result was found in groups treated with Helix aspersa extracts and especially the hydroalcoholic extract. Protein level decreased in BALF. This finding suggests that mollusk could act on signal pathways implicated in inhibiting cytokines, chemokines and lipid mediators (TNFα, leukotriene and prostaglandin) production. Moreover, the microscopic examination of the histological sections of the lungs showed a normal structure of the bronchial wall with the absence of granuloma, especially in groups treated with the aqueous and hydroalcoholic extracts. The administration of Sephadex caused an increase in the weight of the lungs because of the flow of fluid therein. This result is similar to that obtained by Evaldsson et al. (2011). Treatment with NSAID appears to aggravate edema in the lungs. In contrast, edema decreased after treatment with aqueous and hydroalcoholic extracts. Edema is the extravasation of a fluid rich in plasma proteins, causing swelling. This is due to increased vascular permeability during the initial phase of inflammation. Helix aspersa extracts could reduce vascular permeability in lung tissue by acting on inflammatory molecules produced in the lung by inflammatory cells (epithelial cells, neutrophils, macrophages, eosinophils). No change in the group that received the organic extract compared to rats in lot Sx.

Intending to confirm the inflammatory reaction in the lung, verify the involvement of neutrophils and understand the anti-inflammatory mechanism of Helix aspersa extracts, we measured the activity of myeloperoxidase in BALF. Myeloperoxidase is the major constituent of neutrophil granules. It is a heme enzyme with peroxidase and chlorination activity. It uses H2O2 to produce hypochlorous acid (HOCl), a strong oxidant. It has bactericidal and cytotoxic activity. Our results showed an increase in enzyme activity in Sx group BALF. Treatment with aqueous, hydroalcoholic and organic extracts resulted in a decrease of this enzyme activity in BALF. The use of a nonsteroidal anti-inflammatory drug also decreased it. The elevation of MPO concentration increases in alveoli in Mebirouk Romeila and Naimi Dalila, Int. J. Res. Pharm. Sci., 2021, 12(4), 2557-2565
correlation with oxidizing activity in cells and BALF, with the number of neutrophils and with other products as elastase (Mathy-Hartert et al., 2000). In addition, the MPO inhibits anti-proteases, allowing elastase to pass into the bronchoalveolar fluid. It also decreases antioxidant defences (Winterbourn and Brennan, 1997). The increase in MPO activity could also be due to the production of ROS by lung cells under the effect of Sephadex. Decreased MPO activity in the groups treated with Helix aspersa extracts could indicate that they restored a balance between oxidants and antioxidants in the lung tissue. The polyphenols of Helix aspersa crude extract could modulate this MPO activity. It is also believed that this decrease is due to the decrease in neutrophil activity in BALF by inhibiting the production of elastase or by inhibition of their adhesion to the endothelium and their trans-endothelial migration. The hemorrhagglutinating activity of Helix aspersa demonstrated in our study suggests the presence of lectins. This test is only a preliminary study; it must be confirmed by the purification and the structural study of the various possible lectins of this species. The hemorrhagglutinating activity of Helix aspersa had already been demonstrated, and lectins were purified and characterized (Pietrzyk et al., 2015). Lectins could play a crucial role in inhibiting the adhesion and diapedesis process by interacting with adhesion molecules expressed on endothelial cells such as E-selectin. Lectins are proteins or glycoproteins that have a wide distribution in animals, plants and microorganisms. In terrestrial or marine invertebrates, these proteins are often part of an innate immune system and have specificities for carbohydrates present on the surface of pathogenic organisms. Several effects have been demonstrated: anti-inflammatory effect, leukocytes migration, antiproliferative effect, anti-tumor effect and immunostimulatory effect (Zhang et al., 2009; Bachelet, 2013). Lectins are not the only biomolecules known for their biological activity, there are other types of molecules that have been tested for their inflammatory, antioxidant and antiproliferative activities, in particular lipids, proteins, polyphenols, sugar and sulfates. Helix aspersa is also rich in those bioactive molecules and the results of a subsequent study confirm this, (Kouachi et al., 2017).

CONCLUSIONS

Our results showed that extracts of Helix aspersa pulmonary inflammation. To our knowledge, no preliminary study hasn’t yet evaluated the anti-inflammatory effect on the lung of the various extracts of this gastropod. The presence of several biomolecules in Helix aspersa foot like proteins, enzymes, polyphenols, flavones and minerals could be responsible for biological effects as it happens anti-inflammatory effect. The administration of aqueous, hydroalcoholic and organic extracts (1 mg/mL) in rats with pulmonary inflammation showed anti-inflammatory activity probably due to decreased total leukocytes in the blood and the fluid bronchoalveolar. Both aqueous and hydroalcoholic extracts decreased edema. These extracts also induced a decrease in the level of inflammatory molecules in the bronchoalveolar fluid.

In lung tissue, we showed that the three extracts decreased the infiltration of inflammatory cells into the pulmonary alveoli caused by Sephadex particles. They inhibited tissue degradation by modulating neutrophil activity. The extracts reduced the size of the granuloma caused by Sephadex. The three extracts have an antioxidant effect on the lung by decreasing the neutrophils activity, which would result from the inhibition of the myeloperoxidase activity.

ACKNOWLEDGEMENT

We thank ZETTAL Hosna, Dr MEROUANE Fateh and KAID Narimène for their technical assistance in MPO assessment in the BALF, the hemorrhagglutinating activity of the extracts and histological investigations.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding Support

This work is a part of the research project (code: PRFU / D000L05ES250220180002), approved by the Algerian Ministry of Higher education and Scientific Research. The authors are grateful to the Algerian Ministry of Higher education and Scientific Research for the financial help.

REFERENCES


Bradley, P. P., Priebat, D. A., Christensen, R. D., Rothstein, G. 1982. Measurement of Cutaneous Inflam-


