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In-vivo anti-inflammatory effects of Roman Chamomile (*Chamaemelum nobile*) aqueous extracts collected from the National Park of El-Kala (North-East, Algeria)

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ABSTRACT

The aim of our study is to evaluate anti-inflammatory effect of *Chamaemelum nobile*. Aqueous extracts were administrated to Wistar rats in bronchial-inflammation experimentally induced by an allergen and ovalbumin, administered intraperitoneally / intranasally (20mg/kg/day). Experimentation showed disturbances in bronchoalveolar fluid with increased leukocyte and lymphocyte levels as well as IL-4 concentration in the lungs and erythrocytes associated with high lipid peroxidation. There were disturbances in enzymatic and non-enzymatic antioxidant defense system. Lungs histopathological showed an inflammatory lymphoplasmacytic infiltrate, moderate edema of alveoli, vascular congestion and suffusion hemorrhage. Administration of aqueous extract to OVA-sensitized rats caused a significant and very highly significant improvement of MDA levels in lungs, erythrocytes, GSH, GPx, GST, catalase and SOD. We notice a decrease in IL-4 in LBA and lungs alongside reduced inflammatory cell infiltration, mild bronchiolar dilation, mild alveolar edema and normal cell morphology allowing us to conclude on the effectiveness of anti-inflammatory activity of Roman chamomile.

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Introduction

Inflammation is a complex process that occurs in response to physical, chemical or infectious attacks (1). Most often, this reaction is beneficial for the attacked organism. It involves many biological systems that aim to destroy or eliminate foreign substances. Sometimes, inflammation can be harmful due to the aggressiveness of the pathogen and its persistence. It is accompanied by pain, redness, heat, increased vascular permeability, protein denaturation and destruction of cell membranes (2). Bronchial asthma is an inflammatory disease of the lower airways, which constitutes a public health problem affecting over 300 million people worldwide (3). Clinical manifestations of asthma include wheezing, shortness of breath, chest tightness, and coughing and are triggered by several factors such as exposure to aero-allergens, tobacco smoke, physical exercise and respiratory tract infections. Major pathophysiological mechanisms that characterize this pathology are bronchial hyperreactivity (HRB), reversible airway obstruction and inflammation dominated by eosinophils and mast cells (4). The concept of oxidative stress is found in type I hypersensitivity reactions, in which the inflammation of the airways is caused by the production of reactive oxygen species (ROS) that are secreted by inflammatory cells (5). These ROS can be directly responsible for the lung lesions and the contraction of the bronchial muscles, participating in the bronchial obstruction typical of asthma. An anti-inflammatory is a substance intended to fight inflammation (natural or chemical). Chemical anti-inflammatories are divided into steroidal (corticoids) and non-steroidal (NSAIDs) anti-inflammatory. In addition to their significant side effects (headaches, dizziness, nausea, allergies, etc.), their main limitation is that they have a symptomatic and non-curative action. In other words, they act on the symptom and not on the cause. Because of the reputed ineffectiveness of drugs currently used to permanently cure asthma and the fear of their known side effects, there is a pressing need to find new natural therapies (5). In this regard, natural products and herbal remedies used in traditional medicine have been the source of many medicines. Many medicinal plants exhibit interesting biological and pharmacological activities and are used as therapeutic agents (6). Particularly, the use of plant extracts helps in reducing the phenomenon of oxidative stress in respiratory system diseases. Chamomile is an example of that. It can be used in the form of infusion, ointment, lotion, and inhalation. Appreciated for their anti-inflammatory properties, there is a great diversity of taxa. We were interested in Roman chamomile (*Chamaemelum nobile*) which is widely distributed throughout the northern periphery of Algeria. This perennial herbaceous plant (hemicryptophyte), aromatic and medicinal bitter tonic, stomachic, antispasmodic and sedative is recognized as a universal remedy for its healing and biological properties (7). Several clinical studies have confirmed the effectiveness of treating cha-

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momile, as an analgesic (8), an anti-inflammatory and antioxidant (9), an antibacterial (10), a broncho-relaxant (11), in the treatment of polycystic ovaries (Pcos) (12), and in the treatment of diabetes (13). The purpose of this study was to confirm the effectiveness of the aqueous extract of chamomile against oxidative stress on an experimental asthma model induced by ovalbumin.

Materials and Methods

Harvest and collection of plant material

An endemic plant was used, namely, Roman chamomile (*Chamaemelum nobile*). In the form of an aqueous extract, it was used on rats of the Wistar strain that were sensitized via an experimental asthma protocol. We find this plant on the edges of roads, grassy moors, and lawns, on light and sandy soils in eastern Algeria. The harvest of Roman chamomile was carried out in April, May and June 2018 at the level of the department of El-Tarf, in the district of Bouteldja. Harvesting was done by hand according to good harvesting practices for medicinal plants. Once picked, the plants were left to dry in the open air in a room with permanent ventilation, allowing the humidity to be extracted from the plant for a period of two weeks before being ground into powder and stored away from moisture and light until its subsequent use.

Preparation of the aqueous extract

The extraction method used was adapted from the data. Ten 10 grams of the crushed plant were macerated in 100 ml of distilled water, and stirred for 24 hours; Then, these aqueous extracts were filtered and kept in the fridge for later use.

Animals and breeding conditions

28 male Wistar rat strains were involved in this study. The rats were obtained from the Pasteur Institute (Algiers, Algeria). The experiments on these animals were carried out in accordance with the guidelines for the ethics of animal experiments. These rodents, aged from 6 to 8 weeks and with an average live weight of 200±10 g, were subjected to a period of adaptation, under the conditions of the animal facility, at a temperature of 25°C and a natural photoperiod (light cycles/12 h obscurity). They were reared in polyethylene cages that were lined with litter. The cages were cleaned and the litter changed every day until the end of the experiment. They were fed with an energy-balanced concentrate from the ONAB factory of Bejaïa (Algeria). The food was weighed every day as well as the amount of water consumed. This allowed us to calculate the evolution of body weight in the different groups.

Group formation and rat treatments

The rats were divided equally into 4 groups of 6 rats, each designed for the study of inflammation (experimental asthma). They were sensitized to ovalbumin and/or treated with the aqueous extract of the Roman chamomile plant. The 4 groups consist of:

- The first group consisted of control rats one (T) that had undergone the same treatment as the other groups but only received 0.9 % saline solution.
- The second group consisted of rats that were sensitized to ovalbumin (OVA) at 1 ml/1mg, developing experimental asthma (14,15).
- The third group was treated orally by gavage with 20 mg/kg of the chamomile extract (16).
- The fourth group consisted of rats that were sensitized to OVA and treated with a dose of 20 mg/kg in chamomile extract at 1 mL/kg/day.

Sensitization and exposure to aerosols

Sensitization and exposure to aerosols were carried out according to the method described by Khaldi et *al.* (17). Subject rats were immunized on the first and fifteenth day by intraperitoneal injection of 10 mg of ovalbumin (Ova) that is adsorbed to 1 mg of aluminum hydroxide in a volume of 1 ml of phosphate-buffered saline (PBS). On days 21, 22, 23, 24, and 25, the rats were placed in a plexiglass exposure chamber that was connected to the outlet of an ultrasonic aerosol generator (OMRON, NE-C29-E) for 30 minutes (18).

Ovalbumin provocations

The ovalbumin inhalation method was performed as described by Chekchaki et *al*. (19). 100 mg of $Al(OH_3)$ was dissolved in 100 ml of a 0.9 % saline solution then another 500 mg of ovalbumin was also dissolved in this same solution which was inhaled afterward through the use of an aerosol from day 21 to day 25. The last exposure to the aerosol was carried out 72 h before the end of the experiment. The animals in the other groups were challenged with 0.9 % saline solution. The sacrifice was through decapitation and took place on day 28, 72 hours after the last inhalation of ovalbumin.

Sample preparation

Blood is immediately collected in two types of tubes. The first dry is intended for centrifugation at 3000 rpm for 15 minutes. The collected serum is used for the blood parameters. As for the erythrocyte pellet, it will be used for the preparation of erythrocyte lysates which were intended for oxidative stress parameters. The second tube, containing an anticoagulant (EDTA), was intended to measure hematological parameters (blood count formula). Sampling of the broncho-alveolar fluid is carried out using the sheath of a micro-infuser (Epicranial device with PVC tubing) whose dimensions are: 0.8 mm × 19 mm × 300 mm ("Sharp" lot 20070722. Medico-Surgical Industries Rouiba-Algiers). The trachea was cannulated and bronchoalveolar lavage (BAL) was performed with 1 mL of PBS solution 3 times in succession (17). BAL fluid was collected and then centrifuged at 1500 rpm for 5 min at 4°C. Once the supernatant had been recovered and stored for the total protein assays and IL-4 evaluation, the cell pellet was resuspended in 1 mL of PBS solution for cell counting, using a hemocytometer. Organ harvesting was done quickly after sacrifice. Animals were dissected for organ harvesting (lungs). These organs were subdivided into two parts: the first half is stored in the freezer for the subsequent preparation of the homogenate, which was used to assay the oxidative stress parameters; the other half is fixed in a 10% formalin solution in order to make histological sections.

Preparation of erythrocytes and homogenates

The preparation of the homogenate was carried out according to the method written by Rouag et *al.* (20). The lungs were quickly removed, washed in 0.9% NaCl solu-

tion and weighed after careful tissue removal. A quantity of 1 g was homogenized in 2 ml of phosphate buffer solution (TBS: 50 mM Tris, 150 mM NaCl, pH 7.4) at 1:2 (w/v), under ice-cold conditions. The homogenates were centrifuged at 3000 rpm for 35 min at 4°C; the supernatants were aliquoted and stored at -20°C. The sediment containing the erythrocytes was suspended twice in a saline solution of phosphate buffer [KH_2PO_4 (10 mM), NaCl (150 mM), pH 7.4], and centrifuged at 3000 rpm for 15 min at 4 °C for the first wash; and at 4000 rpm for 30 min at 4°C for the second wash. Hemolysates were then aliquoted and stored at -20°C prior to use for oxidative stress parameters.

IL-4 assay

The interleukin-4 (IL-4) assay was carried out according to the method described by Khaldi et *al.* (15). IL-4 is measured in the bronchoalveolar fluid and the crushing supernatant of the lungs by the Enzyme Linked Immuno-Sorbent Assay (ELISA) method. This immunological test is conventionally used for protein detection and assay according to the protocol of the commercial kit (Rat Interleukin-4 platinum ELISA kit (BMS628/BMS628TEN)). The first step was already carried out when the strips were purchased, and is called "coating". During this step, the anti-IL-4 antibody was attached to the bottom of the wells (this step lasted 24 hours at 4°C). The second step corresponds to the binding of IL-4 which will be demonstrated in the different biological fluids.

Preparation of the range

Eight wells of the plate were used to produce the standard curve at different concentrations. The rat IL-4 standard was then reconstituted by adding distilled water and letting it stand for 10-30 minutes. After that, it was mixed gently to ensure complete and homogeneous solubilization. A series of seven dilutions (C1: 100 pg/ml; C2: 50 pg/ml; C3: 25 pg/ml; C4: 12.5 pg/ml; C5: 6.25 pg/ml; C6: 3.12 pg/ml; C7: 1.56 pg/ml) was prepared from the standard solution of IL-4 at the concentration of 200 pg/ml.

ELISA steps

The ELISA method was performed as described by Shun-Ming Ting et *al.* (21):

- 50µL of standard diluent Buffer with 50µL of each sample (BAL/lung) is added to all wells of the plate. Then, 50 µL of the biotin-conjugated anti-IL-4 antibody solution was added to all the wells. After homogenization, the plate is incubated for 2 hours at room temperature and washed three times with the washing solution.
- 100 µL of the Streptavidin-HRP solution was added to each well. After homogenization, the plate is incubated for one hour at room temperature and washed three times with the washing solution.
- 100 µL of stabilized chromogen (TMB) was added to all the wells which resulted in turning the color of the mixture to blue. The plate is then incubated for 10 min at room temperature and in the dark.
- 100 µL of stop solution is added to all wells. Adding this solution will cause the color to change from blue to yellow.

Reading and determination of concentrations

The absorbance reading is then taken at 450 nm (Mindray MR-96A Reader). The intensity of the color is directly proportional to the concentration of IL-4 which is determined by comparison with the reference range of the standard, expressed in pg/mL.

Protein assay

This assay was conducted following the method described by Djaber et *al*. (22). 0.1 ml of homogenate or standard (BSA) was mixed with 5 ml of Coomassie blue. After 5 minutes of incubation, the reading of the optical densities against the blank was carried out at 595 nm. The protein concentration was determined by comparison with a standard range of BSA (1 mg/ml), previously carried out under the same conditions.

Determination of lipid peroxidation through measurement of malondialdehyde (MDA) levels at the tissue level

MDA is one of the end products formed during the free radical-mediated breakdown of polyunsaturated fatty acids. It is the most widely used marker in lipid peroxidation, particularly due to the simplicity and sensitivity of the assay method. The MDA assay is carried out according to the method described by Djaber et *al.* (23). The principle of this assay is based on the condensation of MDA in an acidic environment medium and in hot conditions with thiobarbituric acid (TBA). The reaction results in the formation of a pink-colored complex between two molecules of TBA, which can therefore be measured at 530 nm.

Determination of reduced glutathione level

The dosage of reduced glutathione (GSH) is carried out according to the method described by Sekiou et *al.* (24). The reaction consists of cutting the 5,5'-dithiodis-2-nitrobenzoic acid (DTNB) molecule with GSH, which releases thionitrobenzoic acid (TNB) to develop a yellow color. The resulting product is reduced by glutathione reductase to recycle GSH and produce more TNB. The rate of production of TNB is proportional to the concentration of GSH present in the sample. The measurement of the absorbance of the TNB was carried out at 412 nm.

Determination of glutathione peroxidase enzymatic activity

Glutathione peroxidase (GPx) catalyzes the oxidation of reduced glutathione (GSH) by cumene hydroperoxide. In the presence of GSH reductase and NADPH, oxidized GSH is immediately converted to its reduced form with concomitant oxidation of NADPH--NADP+. The enzymatic activity of GPx was measured by the method described by Amraoui et *al.* (25). This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of GSH. Glutathione peroxidase activity was measured at 420 nm.

Assay of the enzymatic activity of catalase

The activity of CAT was measured according to the method described by Salma et *al*. (26). The reaction mixture of 1 ml contained a 780 ml phosphate buffer (100 mM, pH7), 200 ml H_2O_2 (500 mM) and 20 ml supernatants. The reaction started by adding H_2O_2, and its decomposition was monitored by following the decrease in absorbance at 240 nm for 1 min.

Assay of the activity of glutathione-S-transferases (GSTs)

The glutathione S-transferase (GST) activity was determined by the method described by Salma et *al.* (26). The reaction mixture of 1 ml contained 0.830 ml of phosphate buffer pH 6.5 (0.1 M), 0.05 ml of CDNB (0.02 M), 0.1 ml of GSH (0.1 M) and 0.02 ml of the homogenate was then read their increase in the absorbance every minute during the 5 min at 340 nm.

Assay of superoxide dismutase activity

The activity of superoxide dismutase (SOD) is determined by post mitochondrial fraction (S9) of homogenate from its ability to inhibit the reduction of nitro blue tetrazolium (NBT). Thus, the reaction medium contains a photosensitizing molecule (riboflavin) as well as NBT, a molecule capable of being reduced by O_2 generated by riboflavin. The intense lighting of the reaction medium induces the oxidation of the latter which yields an electron to the oxygen to form a superoxide anion that interacts with the NBT (14).

Histopathological examination

The formation of the histological sections was carried out according to the method described by Berrouague et *al.* (27). The lung was dissected and immediately fixed in a 10% formalin solution, treated with a series of graded ethanol, and then embedded in paraffin (increased at 56/58°C). Paraffin sections were sliced 5 µm thick using a microtome (Leica RM2125RT), followed by hematoxylin and eosin staining. Finally, the sections were observed, analyzed under an optical microscope, and then photographed.

Statistical analysis

Values were presented as the means ± standard error mean (SEM) of the six rats in each group. The significant difference between groups means' was determined by one-way ANOVA followed by Tukey's test using GraphPad Prism 8.0.2. The statistical significance of the difference was taken as $p<0.05$.

Results

The variations in the body weight of the rats and in the relative and absolute weight of the lungs are presented in Table 1. Sensitization by OVA induces a decrease in body weight as well as a very highly significant ($p <0.001$) increase in the absolute and relative lung weight values compared to control rats. The administration of the aqueous extract (EA) of *Chamaemelum nobile* to sensitized rats allowed a return to normal of the absolute and relative weight significantly ($p <0.05$) of the lungs compared to the OVA group.

Effects of plant treatment and OVA sensitization on leukocyte lineage

The leukocyte cell count in the bronchoalveolar fluid (BAL) is shown in Table 2. Rats sensitized to ovalbumin had a very highly significant decrease in total cells, leukocytes and monocytes. A very highly significant increase in lymphocytes and granulocytes in comparison with control rats is also noted as well as a highly significant decrease in the monocyte rate of rats treated with EA in comparison with control rats. The treatment of rats with EA finally resulted in an improvement that reads as a very highly significant increase in the number of total cells, granulocytes, and in a highly significant number of monocytes compared to the OVA group.

Total protein assay

The dosage of total proteins was carried out in the LBA liquid represented in Table 1. The results show that sensitization to OVA causes a very highly significant increase ($p < 0.001$) in the total proteins compared to the control group. The administration of the plant decreased the total proteins compared to the control group.

Variation of oxidative stress parameters in lungs and erythrocytes

The inflammatory syndrome is generated by oxidative stress following an increase in the production of reactive oxygen species and a decrease in the cofactors of the an-

Table 1. Variations in body weight, relative weight, absolute lung weight, leukocyte lineage cells in bronchoalveolar fluid (BAL).

Parameters	Control	OVA	EA	OVA /EA
Original weight	272.30 ± 31.15	233.80 ± 25.65	253.00 ± 26.68	241.00 ± 15.54
Final weight	313.80 ± 44.33	288.00 ± 39.88	284.30 ± 23.55	279.80 ± 25.21
Weight gain	41.50 ± 32.46***	54.17 ± 22.51***	31.33 ± 11.96***	38.83 ± 27.79**
Absolute lung weight	02.36 ± 00.15	03.05 ± 00.08***	02.50 ± 00.39##	02.71 ± 00.21
Relative weights	00.83 ± 00.04	01.03 ± 00.03***++	00.87 ± 00.07	00.90 ± 00.11#
Total cells ($x10^3/\mu l$)	01.50 ± 00.31	00.73 ± 00.16***	01.85 ± 00.20# # #	01.31± 00.14# # #+ +
Leukocytes($x10^3/\mu l$)	00.78 ± 00.14	00.41 ± 00.11***	00.71 ± 00.11# #	00.51 ± 00.14*
Lymphocytes %	35.90 ± 01.79	43.61 ± 02.40***	36.48 ± 01.10# # #	41.65 ± 00.97***+ + +
Monocytes %	10.72 ± 01.34	03.67 ± 00.12***	08.92 ± 00.44**# # #	05.46 ± 00.23***# #+ + +
Granulocytes %	52.10 ± 02.88	59.07 ± 01.48***	53.93 ± 01.03# # #	54.43 ± 00.66# # #
Total protein (mg/l)	01.36 ± 00.19	01.86 ± 00.11***	01.46 ± 00.13# # #	01.75 ± 00.11***+

*p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats;

p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats;

+p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

Table 2. Variations in oxidative stress parameters and IL-4 level in the lungs.

Parameters	Control	OVA	EA	OVA /EA
MDA (nmol/mg prot.)	01.65 ± 00.37	02.88 ± 00.53**	01.43 ± 00.33# # #	02.09 ± 00.61#
GSH (µmol/mg prot.)	00.81 ± 00.08	00.30 ± 00.20***	00.71 ± 00.08# # #	00.53 ± 00.16*+
GPx (µmol GSH/mg prot.)	01.56 ± 00.33	00.59 ± 00.33***	01.14 ± 00.38	00.81 ± 00.33**
GST (nmol /min /mg prot)	01.20 ± 00.34	00.59 ± 00.07***	01.03 ± 00.03 # #	00.76 ± 00.12**
CAT (µmol H2O2/mg prot.)	151.50 ± 30.02	76.51 ± 14.17***	140.80 ± 27.43# # #	92.64 ± 07.83***+ + +
SOD (UT/mg prot)	176.20± 11.58	80.23 ± 12.43 ***	164.30± 19.44###	134.70± 18.01***# # #+
IL-4 (pg/mL)	03.84±0,29	08.02±01.74**	03.33± 01.23##	07.18± 00.67+

*p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats;
p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats;
+p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

tioxidant enzyme systems. We measured oxidative stress parameters (reduced glutathione, glutathione peroxidase, catalase, superoxide dismutase, glutathione-S-transferases and malondialdehyde) in erythrocytes and lungs.

At the pulmonary level

The results represented in Table 2 and Figure 2 showed a highly significant increase in the rate of lipid peroxidation of malondialdehyde (MDA) in the lungs. We also notice a very highly significant decrease in the non-enzymatic antioxidant activity of reduced glutathione (GSH) as well as in the enzymatic antioxidant activities dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferases (GST), and catalase (CAT) in the lungs compared to the OVA group. The administration of EA allowed a significant reduction in the level of MDA in comparison with OVA-sensitized rats, a significant increase in the level of GSH, a highly significant increase in the level of GPx and GST, and a very highly significant increase in the level of SOD and catalase compared to control groups.

IL-4 levels in the lungs

Results at the levels of the lungs in the Fig 1 showed a highly significant increase in the level of IL-4 in the OVA-sensitized group compared to the control group. Administration of the aqueous extract significantly reduced IL-4 levels in the OVA/EA group compared to the control group.

In the erythrocyte compartment

Table 3 and the Fig 3 displayed a very highly significant increase in the level of lipid peroxidation of malondialdehyde (MDA) in erythrocytes; a very highly significant decrease in the non-enzymatic antioxidant activity of reduced glutathione (GSH) and in the enzymatic antioxidant activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferases (GST) and catalase (CAT) in the OVA-sensitized groups compared to control groups. The administration of EA allowed a very highly significant decrease in MDA at the level of erythrocytes in comparison with the control group, a very highly significant increase in GST, in GPx and a significant in SOD in comparison with the control groups. We notice also a highly significant increase in GSH and a significant increase in GPx in comparison with the ova-sensitized groups. As a result, it is deduced that the administration of the aqueous extract restores the levels of antioxidant activity elicited.

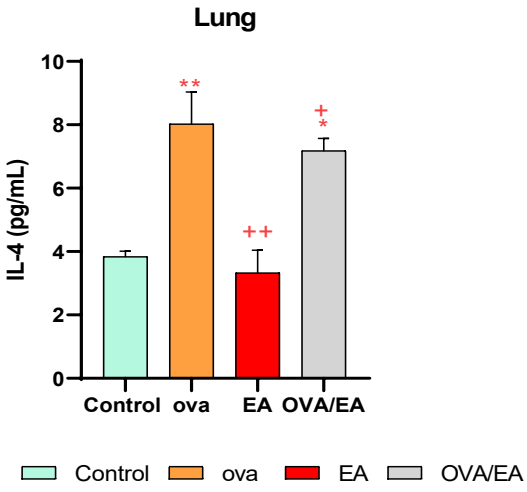

Figure 1. Variation in IL-4 levels in the lungs. *p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats; # p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats; +p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

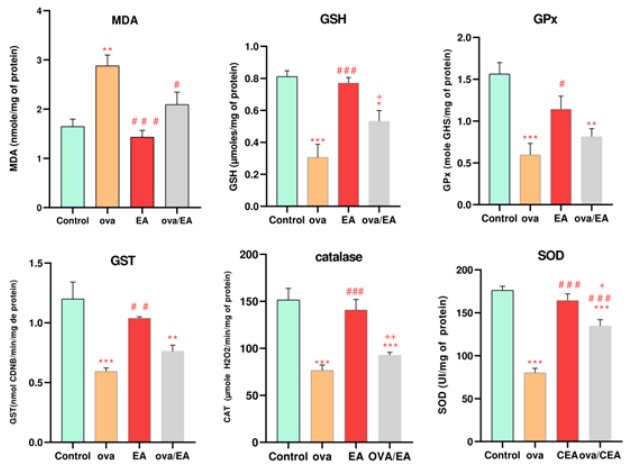

Figure 2. Variations in oxidative stress parameters in the lungs. *p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats; # p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats; +p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

IL-4 level in BAL

BAL results in the Fig 4 showed a highly significant increase in the level of pro-inflammatory cytokines (IL-4) in the OVA-sensitized group compared to the control group. The administration of the aqueous extract significantly re-

Table 3. Variations in oxidative stress parameters in erythrocytes and IL-4 level in BAL.

Parameters	Control	OVA	EA	OVA /EA
MDA (nmol/mg prot.)	00.78 ± 00.21	01.76 ± 00.12***	00.75 ± 00.27# # #	01.66 ± 00.15***+ + +
GSH (µmol/mg prot.)	00.96 ± 00.19	00.34 ± 00.11***	00.84 ± 00.15# #	00.75 ± 00.27# #
GPx (µmol GSH/mg prot.)	01.28 ± 00.20	00.37 ± 00.11***	01.26 ± 00.28# # #	00.79 ± 00.17** #+ +
GST (nmol /min /mg prot)	01.72 ± 00.14	00.50 ± 00.18 ***	01.69 ± 00.09# # #	00.70 ± 00.19***+ + +
CAT (µmol H2O2/mg prot.)	213.30 ± 45.56	41.44 ± 19.30***	167.50 ± 85.50# #	133.30 ± 65.59
SOD (UT/mg prot)	268.70 ± 22.85	195.80± 13.81 ***	256.80± 22,08	225.30± 29.84*
IL-4 (pg/mL)	01.32± 00.28	02.72± 00.45**	00.99± 00.15+ + +	01.40± 00.23# #

*p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats;
p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats;
+p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

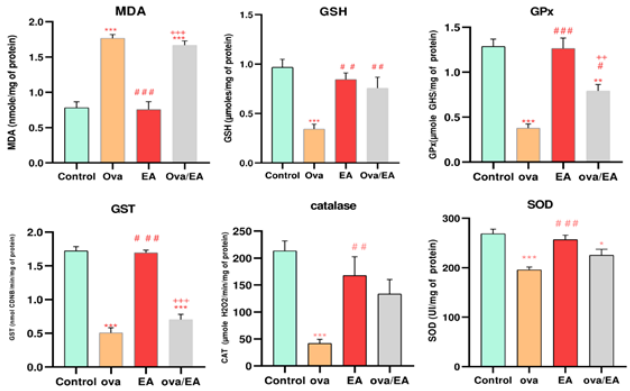

Figure 3. Variations in oxidative stress parameters in erythrocytes. *p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats; # p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats; +p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

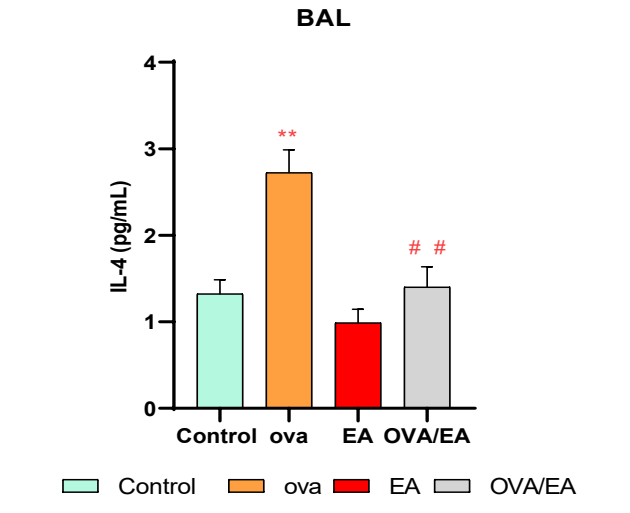

Figure 4. Changes in the level of IL-4 in the bronchoalveolar fluid. *p≤0.05; **p≤0.01; *** p ≤0.001 compared to control rats; # p ≤0.05; # # p ≤0.01; # # # p ≤0.001 compared to ovalbumin-sensitized rats; +p≤0.05; + +p≤0.01; + + + p ≤0.001 compared to rats treated with the plant.

duced the IL-4 level of the OVA/EA group compared to the OVA-sensitized group.

Histopathology results

After sections preparation and their coloring, histological slides are photographed and observed under an optical microscope. In the Fig. 5, we can observe from the top to the bottom and from the left to the right: (a) A normal pulmonary parenchyma is observed for the control rats (T) without any tissular disturbance. On the opposite, and in the treated rats (b) with OVA, there is vascular congestion with hemorrhagic suffusions (blue arrow) and an inflammatory lymphoplasmacytic infiltrate (yellow arrow) of moderate density around the bronchioles with moderate edema of the alveoli. Treatment with EA shows on (d) reduced inflammatory cell infiltration (yellow arrow); mild bronchiolar dilation (green arrow) with mild alveolar edema, and normal cell morphology compared to group (T).

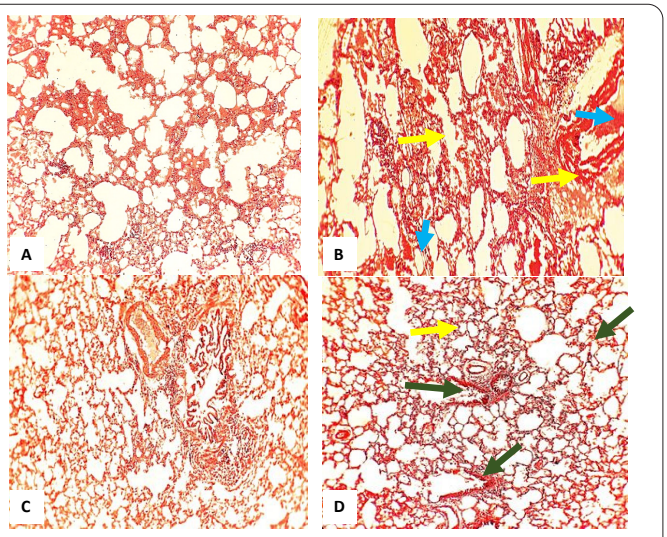

Figure 5. Photomicrographs sections of the lung (x25). (a) control rat. (b) rat sensitized by ovalbumin. (c) rat treated with aqueous extract of camimolle. (d) rat sensitized with ovalbumin and treated with the aqueous extract of chamomile. (blue arrow) represents vascular congestion with hemorrhagic suffusion. (yellow arrow) represents an inflammatory lymphoplasmacytic infiltrate. (green arrow) represents alveolar edema.

Discussion

Inflammation is the vital response of the innate immune system to numerous pro-inflammatory cytokines (IL-4, IL-13 and IL-5) which are involved in directing the inflammatory response through their effects on the immune system. Interleukin-4 (IL-4) is a cytokine that is classified as anti-inflammatory, due to its effects on monocytes and macrophages. It decreases the production of pro-inflam-

matory cytokines such as IL-1, TNF α, IL-6 and IL-8. It allows the induction of T cells in Th 2. The latter acts as a growth factor for Th 2 cells. It is the main mediator of class switching from immunoglobulins (Ig) to isotype E (IgE) by B lymphocytes (28,29). Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and diclofenac are the main drugs capable of inhibiting these inflammatory cytokines and are the first choice for the treatment of inflammation. However, their side effects limit their use (30). Therefore, in recent years, attention has shifted to plants with anti-inflammatory activity such as Roman chamomile (*Chamaemelum nobile*). Since ROS plays an important role in the pathogenesis of certain inflammatory diseases, natural antioxidants can scavenge ROS and improve the inflammatory state and consequently combat these disorders (31,32).

Several studies have been carried out to prove the anti-inflammatory effect of *Chamaemelum nobile*, including Olukayode et *al.* (33) with an *in vivo* evaluation of the analgesic and anti-inflammatory effect of Roman chamomile essential oil (compared to ibuprofen). The latter had been used against inflammation found in the mouse paw. Among the abundant components of the oil are α-bisabolol and farnesene. Their oral administration reduces pain and prevents inflammation in the different test models used in this study.

Another research conducted in 2018 by Tvarijonaviciute showed that the ethanolic extract of *Chamaemelum nobile* is able to inhibit the development of paw edema. Additionally, oral pretreatment with the ethanolic extract significantly reduced the production of pro-inflammatory cytokines, including interleukin (IL-1), tumor necrosis factor (TNF), and IL-6. The ethanolic extract showed a better inhibitory effect on inflammation, which is similar to the effect of the anti-inflammatory drug indomethacin. Thus, *Chamaemelum nobile* is suggested to have an anti-inflammatory effect by inhibiting pro-inflammatory cytokines and reducing oxidative damage. Therefore, this plant can be an alternative to treat inflammation (9).

During a study carried out by Al-Jawad et *al.* (34) to evaluate the broncho-relaxing activity of *Anthemis nobilis* (Chamomile) on 54 patients suffering from chronic bronchial asthma, the tested plant showed a significant elevation in the values of forced expiratory volume in the first second (FEV1%) and forced volume capacity (FVC), with a marked reduction in asthma attacks (34).

Another study (35) was conducted on rodents by experimental induction of polycystic ovary syndrome (Pcos) rodents. This is achieved by the administration of a single dose of estradiol valerate (ev) and a hydro-alcoholic extract of chamomile (*Anthemis Nobilis*). The effect of chamomile extract is observed during histological investigations which showed that the 50 mg/day dose of chamomile decreases cyst size and inflammation.

Chamomile was also used against vulgar psoriasis by Tainá et *al.* (12). It showed the immunostimulating, soothing, analgesic effect of chamomile in a 20-year-old woman, who had the characteristics of psoriasis. Chamomile essential oil containing chamazulene, which is responsible for inhibiting cyclooxygenase (the enzyme that catalyzes the conversion of arachidonic acid into prostaglandins and thromboxanes, one of the responsible for the inflammatory process), confers an anti-inflammatory and healing power. Roman chamomile (*Chamaemelum nobile*) promotes the information of a thin film on the skin leading to an emollient and protective action.

Our study was performed to evaluate the protective effect of the aqueous extract of the Roman chamomile on ovalbumin-induced inflammation and ROS reactive oxygen species production in the lungs and erythrocytes. Ovalbumin stimulation caused an inflammatory state characterized by overweight and increased absolute lung weight. This result is in sync with what was obtained by Mauser et *al.* (36) whose study suggested that provocation with the allergen (ovalbumin) induces increased microvascular infiltration and edema, thus swelling of the inflamed organ.

In our investigation, the relative weight of the lungs has significantly ($p \leq 0.05$) decreased in the OVA/EA-sensitized and treated group, in comparison to the OVA-sensitized group. This implies that the use of the extract reduces tissue damage and edema in the lungs which is believed to be the cause of their increased weight. The leukocyte lineage was disrupted by the use of ovalbumin, showing an inflammatory state that is represented by a very highly significant ($p \leq 0.001$) increase in lymphocytes and granulocytes, in comparison with control rats. In addition, treatment with the aqueous extract has improved the inflammation by restoring lymphocyte levels to their normal values. OVA stimulation has induced a highly significant ($p \leq 0.01$) increase in the level of pro-inflammatory cytokines (IL-4) in the OVA-sensitized group at the level of BAL and lungs in comparison to the control group. The treatment with chamomile extract has significantly reduced IL-4 concentration in comparison to the control and OVA groups, respectively. The reduction in the concentration of IL-4 can be explained by a restoration of the balance between Th1 cytokines and Th2 cytokines (37). This has been suggested by several studies carried out on the bioactive molecules of plants such as flavonoids, which decrease the infiltration of inflammatory cells (25,38). It has been shown that Roman chamomile possesses alpha-bisabolol, alpha-bisabolol oxides A and B as well as matricin (usually converted to chamazulene) and other flavonoids, which possess anti-inflammatory properties. Other studies have shown that azulene compounds have anti-inflammatory and antioxidant properties (39,40), hence the need to study their effects on the oxidative stress generated by the inhalation of the allergen, which in turn contributes to the exacerbation of the bronchial inflammation (41).

Malondialdehyde (MDA) is an end product of lipid peroxidation of the cell membrane that is caused by free radicals derived from oxygen and which can generate an inflammatory reaction (42). The degree of OVA-induced inflammation can be assessed by the accumulation of MDA in the tissues, which determines lipid peroxidation level. In addition, ROS such as hydroxyl radicals, hydrogen peroxide and superoxide anion radicals deactivate antioxidant defense systems, and thus indirectly cause cell damage (43). The antioxidant enzymes SOD, GPx, GST and CAT can protect the cells by eliminating ROS.

Our study proved that *Chamaemelum nobile* extract maintains antioxidant defense systems and does not affect lipid peroxidation since there is no significant variation in the EA group compared to the control one. Sensitization by OVA showed a very high significant increase ($p \leq 0.001$) of MDA levels in the lungs and erythrocytes, and a very high significant decrease ($p \leq 0.001$) of the non-enzymatic antioxidant activity of reduced glutathione (GSH) which

represents one of the many antioxidant defenses which is a factor involved in the protection of various organs against the damages that are induced by oxidants during the inflammatory process (44). Its reduction is due to its high use by GST in the detoxification of H2O2 and the detoxification of active metabolites through the involvement of GPx.

Superoxide dismutase (SOD) is an essential antioxidant that functions as the first line of defense against oxidative stress which catalyzes the superoxide radical into hydrogen peroxide (45). Its decrease leads to impaired antioxidant defense which is a main event in the pathophysiology of hyperreactivity and remodeling, inducing apoptosis and shedding of airway epithelial cells (46). SOD can only work synergistically with the action of CAT and GPx by eliminating the excess hydrogen peroxide formed by it (47). We also noticed a very highly significant decrease ($p \leq 0.001$) in (GPx), (GST) and (CAT) in the lungs and erythrocytes were also observed.

GPx is a key antioxidant enzyme that regulates the level of ROS through its ability to reduce not only hydrogen peroxide but also (hydroperoxides resulting from the oxidation of unsaturated fatty acids) to water. This enzyme could protect cells against damage generated by foreign agents such as ovalbumin (48). According to several authors, the conjugation of GSH to antigenic peptides could be the major pathway for their elimination. It is therefore assumed that glutathione was used for the elimination and detoxification of the administered allergen, which led to a drop in the latter's levels (49).

Treatment with the aqueous extract of chamomile significantly reduces ($p \leq 0.05$) lipid peroxidation and very significantly increases ($p \leq 0.01$) the activity of SOD, GPx, GST and CAT. This may be due to the protective effects of antioxidant molecules, present in the aqueous extract of *Chamaemelum nobile* such as phenolic compounds (flavonoids). The latter has the ability to scavenge free radicals and protect cells against oxidative stress (Kandasamy et *al.* (50) speculate that the antiasthmatic activity of flavonoids is likely due to inhibition of the transcription factor NF-Kβ. Indeed, several other studies have reported the beneficial effect of phenolic compounds on asthmatic symptoms. They reported that the antioxidant activity of polyphenols is related to their ability to chelate ions and remove ROS (51). Therefore, the healing properties anti-inflammatory and antioxidant return to the aqueous extract of chamomile. (52). These properties protect organs and tissues from membrane damage caused by free radicals (53).

At the microscopic level, the damages that are induced by ovalbumin translate into an inflammation that is represented by vascular congestion with hemorrhagic suffusions and an inflammatory lymphoplasmacytic infiltrate of moderate density around the bronchioles. The use of the aqueous extract moderately reduced alveolar edema indicating its efficacy in the treatment of inflammation.

Conclusion

The results of the present study confirm the therapeutic potential of the aqueous extract of chamomile against the pathogenesis of the asthmatic inflammatory process. Our results revealed that the administration of the aqueous extract of *Chamaemelum nobile* reduces the tissue damage of the lungs that is observed on histological sections and also attenuates the harmful effects of oxidative stress by reducing lipid peroxidation, keeping the levels of enzymatic and non-enzymatic defense system antioxidants close to normal, and by decreasing the level of pro-inflammatory cytokines (IL-4), which are responsible for tissue damages. This plant could be a promising alternative in the treatment of allergic asthma.

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Conflict of intrest

Authors reported no conflict of intrest.

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