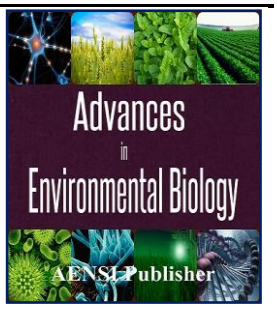
Advances in Environmental Biology, 9(23) October 2015, Pages: 345-351

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/



The Nasal Instillation Effect of the Pollinic Crud Extract of Common Cypresson Murine Model

[1, 2]Bouguenoun Imane, [1, 2]Bensakhri Zinette, [1, 3] Bendjeddou Dalila and [1, 3]Houhamdi Moussa

[1]Laboratory of:Biology, Water and Environment (LBEE), Faculty of nature and life sciences and earth and universe sciences, University of 8 May 1945 - BP 401, Guelma 24000 – Algeria
[2]Department ecology and environmental engineering, Faculty of nature and life sciences and earth and universe sciences, University of 8 May 1945 - BP 401, Guelma 24000 – Algeria –
[3]Department of Biology, Faculty of nature and life sciences and earth and universe sciences, University of 8 May 1945 - BP 401, Guelma 24000 – Algeria –

ARTICLE INFO

Article history:
Received 28 September 2015
Accepted 30 October 2015
Available online 24 November 2015

Keywords:
Allergy, Cypress, Pollinic extract, Nasal lavage, Broncoalveolar lavage.

ABSTRACT

Cypress pollen allergy has increased considerably in the Mediterranean region during these last decades. It is responsible for different symptoms such as rhinitis, conjunctivitis, dry cough and rarely cutaneous manifestations and allergic asthma. Common Cypress or *Cupressus sempervirens* is one of the most widespread species in Algeria which have a very high allergenic capacity (5). In the current approach, we studied how the mice reacted to the nasal instillations of the crude extract of this species. Following sensitizing, the mice leukocyte's formula revealed a decrease in the rate of the neutrophils and an increase in that of the lymphocytes, eosinophil and platelets; however the number of monocytes did not show any difference when compared with control group. As for nasals and broncoalveolar lavage, a significant increase in the cell population was recorded, from where the smears revealed the presence of lymphocytes and rare eosinophils in the treated mice. Also an increase in the number of spleen cells was observed. On the other hand, a histological study of lungs showed major modifications in the structure of the lungsandthusthe inflammatory effect of this crud extract.

To Cite This Article: Bouguenoun Imane, Bensakhri Zinette, Bendjeddou Dalila and Houhamdi Moussa., The Nasal Instillation Effect of the Pollinic Crud Extract of Common Cypresson Murine Model. ***Adv. Environ. Biol., 9(23),*** *345-351, 2015*

INTRODUCTION

Sentinel of the ambient, food and professional environment, the allergy is not a disease. The allergic person expresses an excessive immune reaction to its environment because is born with genetic equipment which characterizes essentially the over-sensitiveness type I where IgE play a major role.

The pollinoses, allergic outward sign induced by pollens, interest classically the ORL spheres (rhinitis and/or conjunctivitis) and pulmonary (asthma), but the cutaneous tables (eczema, urticaria) or oedemas are possible too [25]. That of *the Cupressus* represent a relatively frequent affection in the zones strongly planted in cypress, among other things the Mediterranean zone. It happens essentially in winter but can vary from one year to another according to the climatic factors [6].

Recently, efforts were made to establish the optimal conditions for pollen extraction and to provide a qualitative definition of allergenic components in the extracts. The majority of these researches were accomplished in the Mediterranean Countries where Cupressaceae are more and more significant source of winter allergies such as Italy [3] and Spain [4].

In the present study, we report a description of the effect of the crude extract prepared from one variety of *Cupressus sempervirens* planted in Oued Zenati- Guelma, on an animal model.

MATERIEL AND METHODS

This study was released on *Musmusculus* (BALB/C) female eight week's old coming from pharmacy institute of Constantine, with a body weight between 25 and 40 grams. The handling practiced on these mice is carried out by respecting their wellbeing, excluding any stress condition able to interfere with the results.

Corresponding Author: Bouguenoun Imane, Laboratory of:Biology, Water and Environment (LBEE), Faculty of nature and life sciences and earth and universe sciences, University of 8 May 1945 - BP 401, Guelma 24000 – Algeria

The pollen grains of *Cupressus sempervirens* are collected directly from mature male cones at the region of Oued Zenati, a Commune of Guelma, state in Algeria (36° 18′ 55″ N 7° 09′ 50″ E) during the pollination season.
Preparation of pollen extract

The pollen was obtained following the opening of cypress male cones and a repeated sifting. The contents of the pollen grains are obtained by extraction in a phosphates buffer saline (PBS) 0.01 pH7.2 at 4 °C during 14 hours. The supernatant was collected by centrifugation with 14000g during 1h at 4°C [21] then dialyzed against distilled water and thus filtered using Millipores filters "0.45µm". The filtered solution was frozen and freeze-dried to get finally the crude extract.

Treatment by nasal sensitizing:

12 female mice were divided randomly into two batches, control and treated, which each one contains 6 mice. The mice were sensitized by nasal way by the administration of 100µg of cypress pollen's crude extract (CPE) in a total volume of 10µl of PBS solution in order of 5µl in each nostril at day 0 and day 7. The treatment is repeated with a double dose the 15thand 16th day with 200µg pollen crude extract in a total volume of 20µl of PBS solution in order of 10µl by nostril with a 4 minutes interval. The same treatment was chosen for the control mice by using the PBS only [10].

Nasal lavage:

The 17thday of the treatment, the anaesthetized mice have sustained nasal instillations of 1,5ml of PBS at 37°C in each nostril using a syringe. The liquid collected in the two nasal cavities was centrifuged (800 g at 4°C during 15 minutes) [24]. Each pellet was suspended in 0.9 ml of PBS then, the cells were colored with 0.1ml of trypan blue (0.2%). The results were expressed in leucocytes by microliter of collected fluid.

Bronchoalveolar lavage:

24h after the last treatment, the mice were euthanized. A catheter was introduced into the tracheal tube to realize a double lavage with 0.5ml of PBS at 4°C in order to collect the bronchoalveolar fluid which will be centrifuged with 1500 rpm during 6min. The supernatant was eliminated and the pellet obtained suspended in 0.5ml of PBS [15]. The cells were colored with 0.1ml of trypan blue (0.2%). The results were expressed in leucocytes by microliter of collected fluid.

The blood numerical formula:

The blood collected in tubes with EDTA (acid ethylene diamine tetra-acetic) was intended for the realization of the BNS (blood numerical formula).

Histological study:

After animals' dissection, the spleen and the lungs were isolated and weighed using a balance of precision (Sartorius). The lungs were preserved in formalin 1% and were oriented to the anatomy-pathological laboratory of the hospital "Ibn Zohr - Guelma" for the histological study. The samples were coated out of paraffin and were colored by the HES.

Isolation of spleen cells

The spleen was placed in a Petri box containing 3ml of PBS solution and removed from grease. Using two grips, the capsule was emptied of its cellular contents. The cellular suspension was then filtered and centrifuged during 10 min. at 1500 rpm. The pellet was then suspended in 0.5ml PBS and 4.5ml of red blood cells lysis solution [7]. After an incubation of 10 min, the suspension was centrifuged 10 min/1500 rpm; the pellet is thus suspended in 3ml of PBS, and centrifuged 10 min/1500 rpm. This last step is repeated twice. At the end of the last lavage, the cellular pellet was suspended in 3ml of PBS. The counting of the spleen cells is realized after having diluted 100µl of the suspension in 900µl of trypan blue.

Statistical analysis:

Data were expressed as means ± SD. Statistical significance analysis was determined by using 1-way ANOVA. The differences were considered significant for $P < 0.05$.

Results:

Effect of sensitizing on the leucocytes' formula:

The analysis of the leucocytes' formula revealed a reduction in the total of white blood cells in the treated mice (Tr) by the pollinic extract compared to the control's one (C) (C : 5.57 ± 2.71 $\times 10^3$cell/µl and Tr : 4.17 ± 0.68 $\times 10^3$cell/µl) with a reduction of neutrophils number (C : 2.3 ± 1.4 $\times 10^3$cell/µl and Tr : 0.27 ± 0.007$\times 10^3$cell/µl). However an increase in the rate of the lymphocytes (C: 3.15 ± 1.24 $\times 10^3$cell/µl and Tr: 3.75 ± 0.57 $\times 10^3$cell/µl) was recorded in sensitized mice (fig.1).

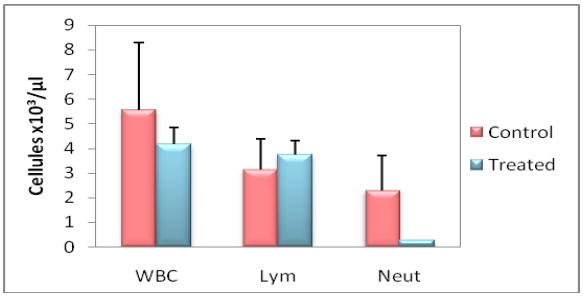


Fig. 1: Variation of the white blood cells, the lymphocytes and the neutrophils number WBC : White blood cells (WBC), Lym : Lymphocytes, Neut : Neutrophils.

The mice were sensitized by nasal instillation with 100 µg of EBP days 0 and 7. A double dose was realized days 15 and 16 with 200 µg of EBP. Blood was recovered for the analysis of the leucocytes' formula. The results are expressed as a mean + / - standard deviation of the two groups. The statistical analysis did not reveal any significant difference.

Regarding eosinophils, an increase (C: 0.07 ±0.02 $\times 10^3$cell/µl and Tr: 0.1 ±0.05 $\times 10^3$cell/µl) was noted,while the rate of monocytes showed no difference between the two batches (0.05 ±0.03 $\times 10^3$cell/µl) (fig.2). The statistical analysis did not reveal any significant difference in comparing the total rate of white blood cells between the two batches.

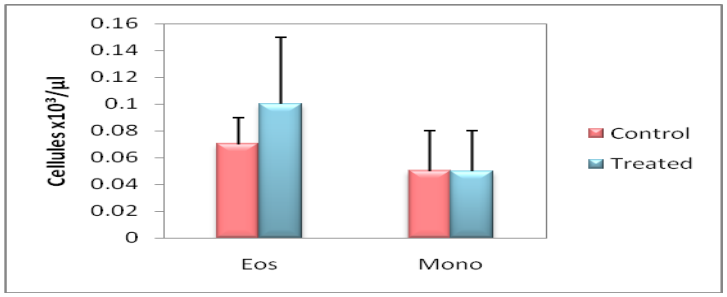


Fig. 2: Variation of eosinophils and monocytes number Eos: Eosinophils, Mono: Monocytes.

The mice were sensitized by nasal way with 100 µg of EBP the days 0 and 7. A double dose was realized days 15 and 16 with 200 µg of EBP. Blood was recovered for the analysis of the leucocytes' formula. The results are expressed as a mean + / - standard deviation of the two groups. No significant difference was recorded.

As for platelets, a noticeable increase in their number was recorded in the sensitized mice (C: 386 ±147$\times 10^3$cell/µl and Tr: 627.75±54$\times 10^3$cell/µl).A significant difference was noted in the treated mice compared with that of the control with P= 0.038 (fig.3).

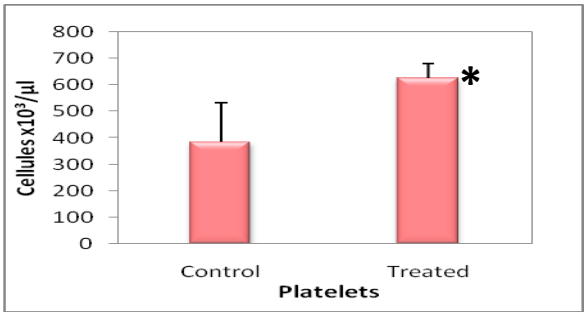


Fig. 3: Variation of the platelets number.

The mice were sensitized by nasal way with 100 µg of EBP days 0 and 7. A double dose was realized days 15 and 16 with 200 µg of EBP. Blood was recovered for the analysis of the leucocytes' formula. The results are expressed as a mean + / - standard deviation of the two groups. A significant difference was found in the treated group while comparing with control group P = 0.038 (* P<0.05)

Effect of pollen extract on the nasal fluid and bronchoalveolar fluid:

After the instillation of the pollinic extract of *Cupressus sempervirens*, a remarkable increase in the number of cells in the nasal fluid was observed (C: 196.25 ±25.15 $\times 10^9$cell/l and Tr: 517.5 ±100$\times 10^9$cell/l). A highly significant difference was recorded with *P*=0.002. The same observation was noted with the number of the bronchoalveolar fluid cells (C: 241.9±58.37$\times 10^9$cell/l and Tr: 333 ±108.23 $\times 10^9$cell/l) (fig.4). On the other hand, the statistical test showed that this difference is non-significant.

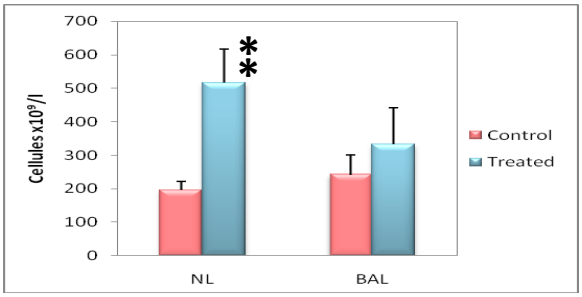


Fig. 4: Cells number in the nasal and bronchoalveolar fluid NL: Nasal lavage BAL**:** Bronchoalveolar lavage.

The mice were sensitized by nasal way (5µl/nostril) the days 0 and 7. A double dose (10µl/nostril) was carried out days 15 and 16. The nasal and bronchoalveolar fluid was recovered after 24h of the last treatment. The results are expressed as a mean + / - standard deviation of the two groups. A highly significant difference was recorded with *P* = 0.002 *(* * P* <0.01)only for the cells number of nasal fluid.

The effect of pollinic extract on the spleen:

The sensitizing of the mice by the pollinic extract of cypress allowed observing an increase in the number of spleen cells (C: 152.5±25$\times 10^9$cell/l and Tr: 304.37±89.02$\times 10^9$cell/l) (fig. 5). The statistical analysis, using the student's t test, revealed only one significant difference relating to the rate of the spleen cells, between the two groupswith *P*= 0.029.

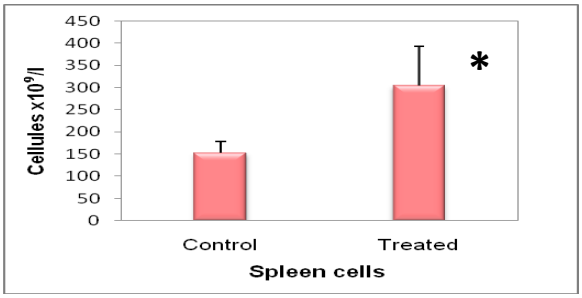


Fig. 5: Effect of sensitizing on spleen cells.

Two groups of 6 mice were used, one treated by PBS and the other by EBP. The mice were sensitized by nasal way (5µl/nostril) the days 0 and 7. A double dose (10µl/nostril) was realized days 15 and 16. The spleen was recovered day 17 and uncapped to count the number of these cells. The results are expressed as a mean + / - standard deviation of the two groups.

The statistical analysis revealed only one significant difference concerning the spleen cells, between the two batches, with *P* = 0.029.

Effect of the sensitization on lungs:

For further confirmation, histological sections of this organ have been made (fig. 6).

The microscopic examination showed in controls, a normal lung with a simple vascular congestion and cells with inter-alveolar partitions. The histological structure seems normal. Indeed, the partitions or cell walls are formed by a whole of cells which pneumocytes and endothelial cells (fig. 6a).

For the mice subjected to nasal instillations with the pollinic extract, a chronic mononuclear inflammatory peri-bronchiolar infiltrate was noticed with presence of lymphocytes (fig. 6b).

The mice were sensitized by nasal way (5µl/nostril) days 0 and 7. A double dose (10µl/nostril) was realized days 15 and 16. The lungs were preserved in formol 1% and were oriented to the laboratory of anatomy-pathological of the hospital "Ibn Zohr - Guelma " for the histological study. The samples were coated out of paraffin and were colored by the HES (Hemaleine-Eosin-Saffran).

The treatment also brings a reduction in the alveolar diameter. This could be due to a thickening of the alveolar wall by proliferation of conjunctive tissue fibers.

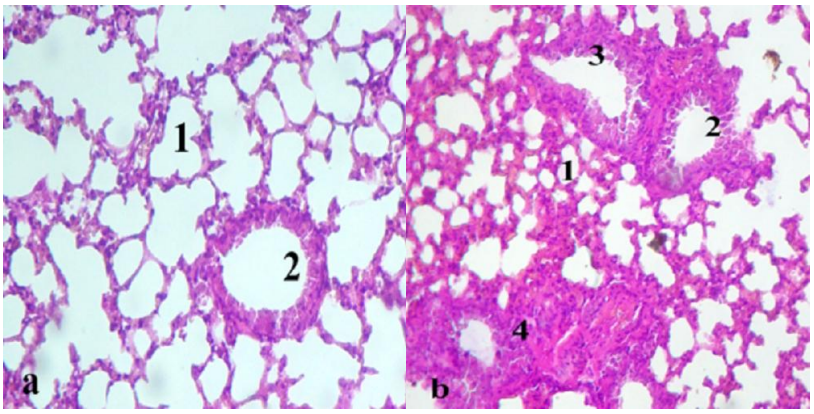


Fig. 6: Histological study of the control and treated mice lungs (x250). a) Normal lung of the witness b) Lung of treated with the extract pollinic 1 – Alveolus, 2 - Bronchiole, 3 - Blood vessels, 4 cellular Infiltrate.

Discussion:

The cypress pollen occupies today one of the first places among all tree pollens considered or deemed able to cause allergic reactions, in particular in the Mediterranean basin. In this context it proves to be interesting to study the effect of the pollinic extract of *Cupressus sempervirens* on the immune system of mice.

The nasal instillation with repeated doses of this pollinic extract allowed to highlight changes in blood parameters which an increase in the number of the lymphocytes, a clinical aspect due to the deposition of pollinic allergens on the mucosal epithelium. These allergens are internalized and treated by antigen-presenting cells.

Indeed, the cells Th_0 differentiate then in lymphocytes Th_2 which also release various cytokines playing a significant role in the allergic reaction and others cytokines which inhibit the activation of the Th_1cells (IL-4, IL-13).These cytokines promote the development of IgE producer B lymphocytes. It has been shown that not only the allergic individuals have in their blood of great quantities of specific lymphocytes Th_2 of allergens, but each one of these cells produces more IL-4 as that of a normal individual (i.e. non-allergic) [20].

Moreover, the decrease in the neutrophils number returns to the fact that these cells represent the first immune cells to migrate from the bloodstream to the site of inflammation [11] this explains the decrease in the white blood cells rate. It should be noted that the increase in vascular permeability allows the migration of neutrophils to the sites of inflammation, also leads to fluid leakage from the vessels and the diffusion of plasma proteins into the tissues and the bronchial mucosa [2]. The trans-endothelial migration is extremely well orchestrated by inflammatory mediators and in dependence of the adhesion molecules expressed by these polynuclear and by endothelial cells [26]. Chemotaxis to inflammatory sites is under the influence of gradient chimioattractants substances [23].

The eosinophils, which are cells with essentially tissue localization, proliferate in response to the IL-5 secreted by the activated lymphocytes Th_2 and migrate under the effect of the same cytokine to the inflammatory sites and the mucous membranes. The increase in their number is observed in the allergic rhinitis, the atopic dermatitis and asthma [5]. The prevalence of the latter is slightly higher for people presenting a hyper eosinophilia [8].

As for the platelets, several lines of evidence indicate their active role in immune reactions, particularly in allergic reactions [13].Following chronic exposure to allergens, platelets induce cause the airway remodeling [17].They have specific receptors for IgE (CD 23) and high affinity receptor FcεRI. The stimulation of platelets via this receptor induces the release of serotonin and RANTES (Regulated on Activation, Normal T expressed, and presumably Secreted) [1],which is the most effective chemotactic factors, selective eosinophils, known until our days, which moreover, induce the degranulation and the preferential release of histamine by the basophils of allergic individuals. Their storage in the platelets underlines their role in allergic inflammatory reactions.

Furthermore, it is important to say that during the effector phase of the hypersensitivity type I there is production of newly formed lipid mediators that are very potent bronco-constrictors, increasing also the vascular permeability.Among these mediators we may mention the Platelets Activating Factors (PAF) which cause the proliferation and the activation of the platelets from where their increase in the sensitized mice [13].

On the other hand, mice intolerance was expressed by the richness of the nasal fluids in leukocytes. This result agrees with released work where it was argued that an increase in the leukocytes cells of the nasal fluid is observed after a sensitizing by the pollinic extract [12].

Other research has shown that the weekly instillation with pollen particles over a long period induced rate increases of the alveolar cells in the BAF (Bronchoalveolar fluid) and a mucus hyper secretion [1], this confirms the results obtained for bronchoalveolar lavage as at the bronchial tree, the mediators induce a bronchoconstriction, swelling of the airways lining and an increase in the mucous membranes secretion [18].

Concerning spleen, it represents a secondary lymphoid organ organized to facilitate the maximum interaction between T and B cells, thus it playing a major role in both humoral immunity and cellular. In allergies case, the cells migrate to the secondary lymphoid organs to present the antigen at CD4 T lymphocytes which are activated leading to the establishment of a TH_2 response, driving therefore, to the activation and the differentiation of B lymphocytes specific to the allergen, that explains the increase in the spleen cells number.

The histological study allowed to visualize an inflammatory aspect in the treated mice by our extract because certain type of allergies, in particular respiratory, are characterized by the accumulation of the inflammatory cells in the airways and the lungs including eosinophils ones and macrophages [16].

Conclusion:

In other term, the sensitizing of the mice by the pollinic extract of cypress (*Cupressus sempervirens*), following nasal instillations, led to the description of different clinical manifestations not only on the cellular level but also on the histological aspect. The aim of this study was to highlight the reaction of the body of an animal model against the allergens of this species, which these results testify the remarkable inflammatory effect.

A thorough study of various allergens, including the major allergen of this species giving rise to a very regular cross-reactivity, as well as stimulating the pulmonary airway by different cytokine is in progress.

ACKNOWLEDGEMENTS

The authors are grateful toMERZOUG SeyfEddine, the staff of the laboratories of Applied Chemistry - University 8 May, 1945 Guelma (Algeria), medical analysis laboratory of Dr. KACI-TIARTI S.from Annaba (Algeria), anatomy-pathological laboratory of the Ibn Zohr Guelma's Hospital and the Institute of Pharmacy – Constantine (Algeria).

REFERENCES

[1] Abou Chakra, O., 2009. Allergénicité des granules cytoplasmiques du pollen, D. S.thesis,Diderot Univ., Paris.
[2] Anceriz, N., 2008. Rôle des protéines S100 dans la migration des neutrophiles au site inflammatoire, D. S.thesis,Laval Univ., Québec.
[3] Ariano, R., C. Mistrello, G. Mincigrucci, E. Bricchi, O. Iannotti, G. Frenguelli, 2006. In Vitro and In Vivo Biological Activities of Old and Fresh Cupressus arizonica pollen.J Investigallergol Clin immunol, 16(3): 177-82.
[4] Arilla, M.C., I. Ibarolla, R. Garcia, B. De La Hoz, A. Martinez, J.A. Asturias, 2004. Quantification of the major allergen from cypress (Cupressus arizonica) pollen, Cup a 1, by monoclonal antibody-based ELISA. Int Arch AllergyImmunol, 134(1): 10-16.
[5] Borges, J.P., 2008.Caractérisation structurale et immunologique d'allergènes alimentaires: les protéines de transfert de lipides de fruits, D. S. thesie, Paul Sabatier Univ.,Toulouse.
[6] Charpin, D., B. Hugues, M. Mallea, M. Thibaudon, J.P. Sutra, M. Ivry, G. Balansard *et al*., 1990. L'allergie au cyprès. RevFr Allergol, 30(1): 21-26.
[7] Daum, J.R., D.M. Shepherd andR.J. Noelle, 1995. Physical interactions and early signaling between Helper T lymphocytes and B lymphocytes.InMethods in Immunotoxicology,Eds., Burleson, G.R., J.H. Dean andA.E. Munson. New-York: Wiley-LissInc, pp: 469–481.
[8] El Kettani, S., B. Lotfi andA. Aichane, 2009. Prévalence de la rhinite allergique en milieu rural à Settat (Maroc). E M H J, 15(1): 167-77.
[9] Espinosa, E. and P. Chillet, 2010. Immunologie. Paris : Ellipses.
[10] Fischer, R., J.R. McGhee, H.L. Vu, T.P. Atkinson, R.J. Jackson, D. Tomé, 2005. Oral and nasal sensitization promote distinct immune responses and lung reactivity in a mouse model of peanut allergy. Americ J Path, 167(6): 1621–30.
[11] Hurtado-Nedeleca, M., P. Dang, R.C. Monteiroa, J. El Benna and M.A. Gougerot-Pocidalo, 2014. Physiologie des polynucléaires neutrophiles humains. Revue Francophone des Laboratoires, (462): 25–38.
[12] Kamijo, S., T. Takai, T. Kuhara, T. Tokura, H. Ushio, M. Ota *et al*., 2009.Cupressaceae Pollen Grains Modulate Dendritic Cell Response and Exhibit IgE-Inducing Adjuvant Activity In Vivo. J Immunol,183 : 6087–94.

[13] Klouche, M., M.H.F. Klinger, W. Kfihnel and D. Wilhelm, 1997. Endocytosis, storage, and release of IgE by human platelets: Differences in patients with type I allergy and nonatopic subjects. J Allergy ClinImmunol, 100(2): 235–241.
[14] Kuna, P., S.R. Reddigal, T.J. Schall, D. Rucinski, M.Y. Viksman and A.P. Kaplan, 1992. RANTES, a monocyte and T lymphocyte chemotactic cytokine releases histamine from human basophils. J Immunol, 149: 636-642.
[15] Li, Y., L. Zhang, Y. Liu, X. Yang and X. Sun, 2010. Establishment of a mouse model of Humulus pollen allergen-induced allergic asthma.J Xi ' an Jiaotong University (Med Scien), 31(5): 562-565.
[16] Ma, W.J., M.J. Bao, J.P. Zhu, H.Y. Yao, Y.C. Xie, Y. Guan, 2012. Oral administration of allergen extracts from mugwort pollen desensitizes specific allergen-induced allergy in mice. Vaccine, 30: 1437–1444.
[17] Pitchford, S.C., H. Yano, R. Lever, Y. Riffo-Vasquez, S. Ciferri, M.J. Rose, 2013. Platelets are essential for leukocyte recruitment in allergic inflammation. J Allergy Clin Immunol, 112(1): 109-118.
[18] Platts-Mills, T.A.E., 2007.Immunologie,Paris: Elsevier Masson.
[19] Rawat, A., A. Singh, A.B. Singh, S.N. Gaur, L. Kumar, I. Roy and P. Ravindrun, 2000. Clinical and immunologic evaluation of Cedrusdeodarapollen:a new allergen from India. Eur J Allergy Clinimmunol, 55 (7): 620–26.
[20] Roitt, I.M. and A. Rabson, 2002. Immunologie médicale: l'essentiel. Paris : Maloine.
[21] Schall, T.J., 1991. Biology of the RANTES/SIS cytokine family. Cytokine,3 : 165-83.
[22] Shahali, Y., A. Majd, Z. Pourpak, G. Tajadod, M. Haftlang andM. Moin, 2007. Comparative Study of the Pollen Protein Contents in Two Major Varieties of *Cupressus arizonica*Planted in Tehran. Iran J Allergy Asthma Immunol, 6(3): 123-27.
[23] Swaney, K.F., C.H. Huang and P.N. Devreotes, 2010. Eukaryotic chemotaxis : a network of signaling pathways controls motility, directional sensing, and polarity. AnnuRevBiophys, 39: 265-89.
[24] Urbain, B., J. Mast, B. Goddeeris, M. Ansay and P. Gustin, 1997. Influence d'une exposition aux poussières sur la composition cellulaire des liquides de lavage nasal et broncho-alvéolaire chez le porc. J RechPor Fr, 29 : 17-22.
[25] Vitte, J., 2012. Cofacteurs dela pollinose. Rev Fr Allergol, 52: 189–93.
[26] Woodfin, A., M.B. Voisin andS. Nourshargh, 2010. Recent developments and complexities in neutrophil transmigration.CurrOpinHematol, 17(1): 9-17.