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ASPECTS REGARDING THE IMMUNITY OF THE LABORATORY MOUSE AFTER CHRONIC ADMINISTRATION OF „*IMUNITATE CU 7 CIUPERCI"*

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ABSTRACT
Natural products are used to prevent and treat large series of affections because of their curative and immunity stimulating proprieties. The study herein focused on the effects of natural product „Imunitate cu 7 ciuperci" produced by Fares Romania, on the immunity of laboratory mice. After a chronic administration of this product for three months, the weight of all animals was determined: for the blood collected from the control group and the experimental group, the total number of leucocytes/mm³ was determined, and, on blood smears, so was the leukocyte formula. After sampling and staining, the blood smears ware examined with the optical microscope; on the blood smears was determined the number of figurative elements of blood: lymphocytes, neutrophiles, eosinophiles, monocytes and basophiles. Weight control data showed that the product does not induce weight gain. Data processing does not evidence significant differences in the total number of leucocytes/mm³ between the two mice groups; as about the leukocyte types, significant differences were found only in the case of eosinophyles. These results do not plead for an immunity stimulating effect on the laboratory mice, which means that more tests are necessary.
KEY WORDS*: immunity, laboratory mice, blood smear, leucocytes*

INTRODUCTION

The human body is permanently in contact with pathogen agents (antigen carriers) or free antigens. The blood performs the immune-biological defense function with non-specific and specific mechanisms with the participation of figurative elements. Immunity can be non-specific (native), carried out by humoral and cellular mechanisms and specific (earned) developed by exposure to agents able to induce an immune response: by trans-placental transmission of antibodies, after an illness, through vaccination, gamma globulins and antitoxin administration (Filimon, 2010). It is well known that natural products do increase immunity, and that is why they are used by people to prevent some affections and to grow the resistance of the body.

Fungi that grow in Romania such as *Ganoderma lucidum, Fomes fomentarius* or mushrooms with an asian origin such as *Lentinus endodes* and which are included in the „*Imunitate cu 7 ciuperci"* product are used in a large series of affections such as viral infections (viral hepatitis, flu, herpes), bacterial and fungal infection, diabetes,

protection against radiations, complementary in the case of tumors, cholesterol, increased number of triglycerides, arterial hypertension, allergy, varicose, stress and chronic tiredness (*„Imunitate cu 7 ciuperci "* prospect)**.**

Other fungal compost were proved to have immunostimulator effect, as fungal polysaccharide (Tirziu, 2009), novel polysaccharide isolated from *Lactarius deliciosus* (Hou *et al,* 2013) and phenolic compounds (Zheng *et al*, 2008). It were also published others experiments on laboratory animals, when the diet was supplemented, like rats (Radhika *et al*., 2012), fishes (Ragap & Khalil, 2012), mice (Sahu *et al*., 2013) or chickens (Paulsi & Dhasarathan, 2011). Several *in vitro* experiments, based on plant extract administration and it's effect are described (Yu & Lee, 2010; Ianovici *et al*, 2010; Lee *et al*, 2012). In our institution other recent experiments on laboratory mice were made (Checiu *et al*, 2003; Checiu *et al*, 2006; Checiu *et al*, 2007; Checiu *et al*, 2008; Huțanu, 2011; Filimon *et al*, 2012).

MATERIALS AND METHODS

This study used a group of ten white male mice belonging to the hybrid line from the first generation F1, a cross between the NMRI and the Swiss line, as well as a control group of six male mice from the stock farm of the Biology Department of the West University of Timişoara. The mice were kept in laboratory conditions: fotoperiod of 12 hours light - 12 hours dark, 17°C temperature and 60% humidity. For 3 months, the mice were administered the „*Imunitate cu 7 ciuperci"* product, made by Fares Orăştie Romania, beginning on December 20th 2012 and until March 20th 2013, with the aim of determine the total number of leucocytes.

Among the fungi which make up the used product (*Ganoderma lucidum, Fomes fomentarius, Coriolus versicolor, Inonotus obliquus, Grifola frondosa, Lentinus endodes and Cordyceps sinensis)* some of them grow in our country and others have an asian origin, all of them being used as remedies for some affections, substituting traditional medicine. The daily dose was established by comparison with the recommended dose for an adult human weighting 70 kg. Thus, an adult male weighting 30 grams received 70 milligrams of substance, mixed with the food given for lunch.

The mice were weighted at the beginning and the end of the experiment and their weight expressed in grams was registered.

After 3 months, the sample collection took place. The mice were anesthetized with Sodium Pentobarbital liquid solution (0,1mL /10 grams of body weight) by intraperitoneal injection. Blood was collected from the inferior vena cava by puncture with a sterile syringe.

Blood smears were done immediately after the collection of blood from each treated male mouse and from each control male mouse, and the remaining blood was introduced in EDTA test tubes. Pools were made from the blood of treated mice and

from that of control mice and they were sent to Bioclinica Timişoara in order to determine the total number of leucocytes.

Blood smears were made with the purpose of studying the morphology of the figurate elements from the leukocyte cell line. The May Grunwald Giemsa stain protocol was used. The blood smears were examined with the Optika microscope, with an immersion objective of 100X. 100 figurative elements from the leukocyte cell line were counted for each blood smear and, as they were identified in the microscope field, the number for each category was noted: lymphocytes, neutrophiles, eosinophiles, monocytes and basophiles.

The data was statistically processed by calculating the average, with standard deviation. Then t parameter was then calculated, which meant the comparison between the average of the experimental group with the average of the control group. The differences between averages were considered significant when the p value from the liberty degree table was lower than 0.05.

RESULT AND DISCUSSION

The medium weight of the mice from the control group was compared with the weight of the mice from the experimental group at the beginning of the experiment (fig.1). It can be observed that the average weight of the control mice is higher than the average weight of the treated mice. This result is not statistical significant. No significant differences existed between the average weight of the control mice group and that of the mice from the treated group at the end of the experiment, as can be seen in the figure 2. No significant differences were found between the weight of the mice from the experimental group in comparison to the initial moment; this shows that the product does not induce weight gain, as shown in figure 3.

By examining the blood smears, the number of figurative elements from the leukocyte cell line was obtained: lymphocytes, neutrophiles, eosinophiles, monocytes and basophiles - the leukocyte formula. According to data obtained and shown in figure 4, it can be observed for both groups of mice that lymphocytes, the first figurative elements involved in the immune response, are in a higher proportion, of 64.3%, for the control group, and 57.4% for the treated group; these are followed by neutrophiles, that have a 25% percent for the control group, respectively 26.8% for the treated group and then by eosinophils, in a percentage of 9.4% for the control group, and 13.4 for the treated group . The percentage of monocytes was 0.8% for the control group and 1.5% for the treated group and, finally, basophiles existed in the lowest percent 0.5% for the control group and 1.1% for the treated group.

For each type of figurative elements from the leukocyte cell line, the value of the average number was compared between the two mice groups.

Therefore, in the case of lymphocytes, represented in the figure 5A, the average with standard deviation for the control group of mice was 64.33±2.66, while for the treated group it was 57.4±2.84. There are significant differences between the

two groups of mice, their number being higher for the control mice ($p>0.01$). As about the neutrophyles (fig. 5B), the average with standard deviation for the control group was 25±2.5 and for the treated group it was 26.8±3.19, so an increase of the number of neutrophyles was induced in the treated mice, but the increase was not statistically significant.

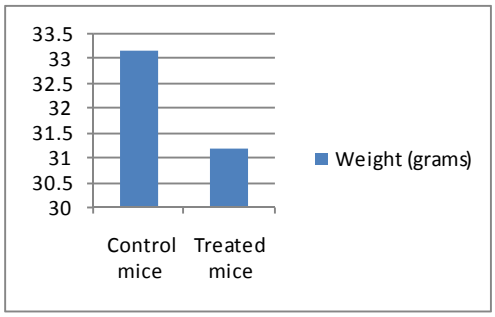

Fig. 1. The medium weight of the mice at the beginning of the experiment



Fig. 2. The medium weight of the mice at the end of the experiment

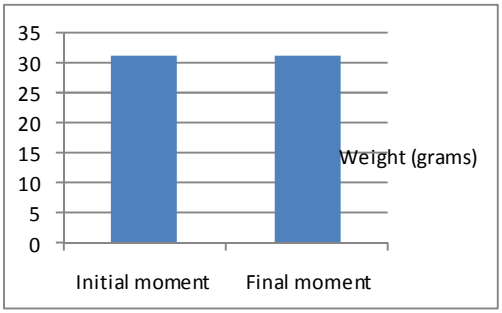

Fig. 3. Medium weight of the mice from the experimental group at the beginning and the end of the experiment

For eosinophiles, the average with standard deviation for the control group was 9.33±2.58, lower than that for the experimental group, which was 13.4±1.89. So

there was a significant increase in the number of eosinophiles for the experimental group, p>0.01 (fig.6). The other types of figurative elements from the leukocyte cell line, monocytes and basophiles, does not present significantly increased values after the treatment. The average with standard deviation for the control group was 0.83±0.75, and for the treated group it was 1.3±1.41 in the case of monocytes (fig.7A.); for basophiles, the average with standard deviation for the control group was 0.5±0.83 and for the treated group it was 1.1±0.57 (fig.7B).

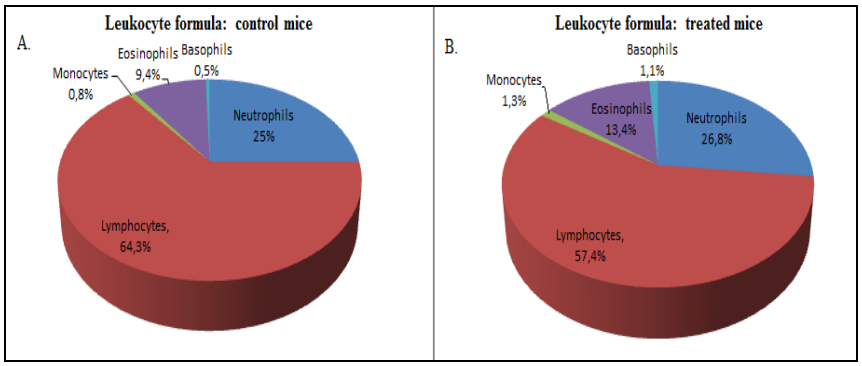

Fig. 4. Leukocyte formula: A-treated mice and B-control mice

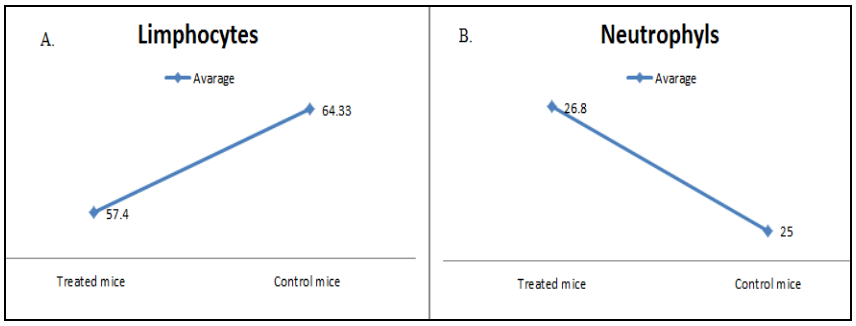

Fig. 5. Avarage of limphocytes (A) and neutrophyls (B) for the control and experimental mice

The total number of leukocytes /mm³ determinated at Bioclinica Timişoara for the two groups of mice was higher in the case of the control mice, 4780 leukocytes/mm³, than the experimental group, witch was 3160 leukocytes/mm³, as we can see from Figure 8. Later, the variation of total number of lymphocytes /mm³ was represented in the charts in figure 9: neutrophiles, eosinophile*s,* monocytes and basophils for the two mice groups, for both cases. For both cases it can be seen that total number of lymphocytes is higher, followed by the total number of neutrophiles and by the other types of leukocytes. This variation coincides with the values obtained within the leukocyte formulas.

There are studies about the effect of some fungi on immunity, but not one based on the effect of fungi combination. There are known studies establishing connections between immunity and different fungi extracts.

According to Chang - Qing Gu *et al*, 2007, an antiviral protein was purified from an extract of *Grifola frondosa* fruit. It was established that this protein, named GFAHP, had an antiviral function, inhibiting *in vitro* the multiplication of the herpes simplex virus type 1 (HSV-1) and its penetration into cells; mice from the BALB line were infested with the herpetic virus, determining different ocular affections like blepharitis, neovascularization and stromal keratitis, which were later treated with different concentrations of GFAHP.

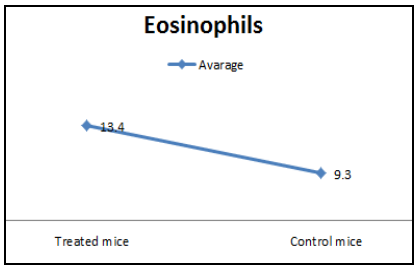

Fig.6. Avarage of eosinophiles for the control and the experimental mice

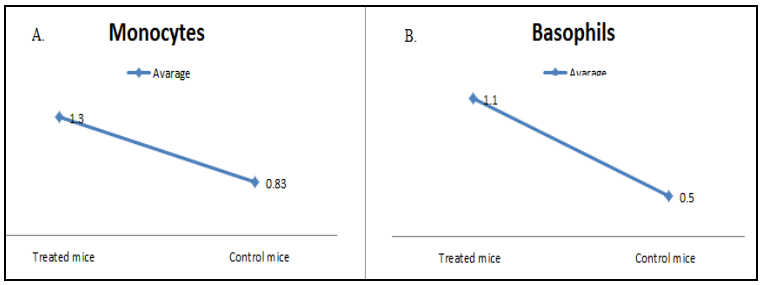

Fig.7. Avarage of monocytes (A) and basophils (B) for the control and experimental mice

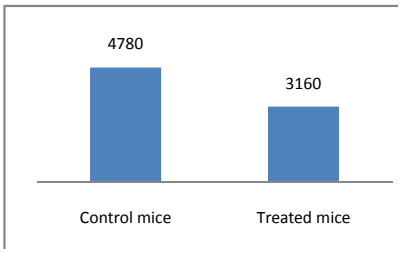

Fig.8. Comparation between the total number of leukocytes /mm³ of the control and the experiment groups

In the Ardeleanu paper (2012), according to the results, there was an erythroblasts increase in the case of the *„Echinacea*" treated embryos two days after

the treatment and an erythrocytes increase for the same embryo, one day after the administration. Average values of the erythroblasts from the other days, in the case of the treated embryos, were approximately equal to those of the control embryos.

The average weight of the treated mice was the same at the beginning and the end of the experiment, meaning that the product does not induce weight gain. *„Imunitate cu 7 ciuperci"* induces a significant increase in the number of eosinophyls, an active involved in the immune response against allergic and parasite reactions; the other figurative elements from the leukocyte cell line did not have significant increases. It is possible that the product mihght causes allimentar allergy or other affections because of the increased number of eosinophils as result from other studies (Faur et. al., 2001; Ianovici, 2007; Ianovici, 2008; Simon et. al., 2010; Alfadda, 2010; Ianovici *et al*, 2011; Van Gramberg, et. al.,2013).

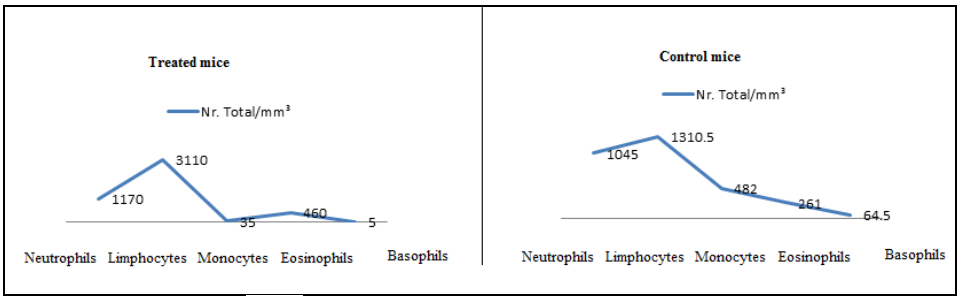

Fig.9. Variation of total number of leukocytes /mm³

CONCLUSION

The data obtained in this experimental study focusing on the leukocyte formula does not evidence an immunity stimulating effect for the laboratory mice. We believe that other investigations are needed before we can affirm with certainty that product *„Imunitate cu 7 ciuperci"* does not have an immunity stimulating effect on laboratory mice.

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REFERENCES

- Alfadda A.A., Storr M.A., Shaffer E.A. 2010. Eosinophilic colitis: epidemiology, clinical features, and current management, *Therapeutic Advances in Gastroenterology*, 4 (5): 301309
- Ardeleanu A. M. 2012. Study on the influence of *Echinacea* extract upon the development of blood cells at young chick embryo, *Annals of West University of Timişoara, Ser. Biology*, 15: 27-*38*
- Chang-Qing, G., Chao, F., Jun-Wen L., Min, J., Wang, X. W., Zhi-Qiang, S. 2012. Isolation, identification and function of a novel anti-HSV-1 protein from *Grifola frondosa, Antiviral Research,* 75: 250–257
- Checiu I., Checiu M., Checiu D., Capalnasan I., Tuduce I. 2003. Investigations of teratogenic effects induced by copper upon early postimplantational mouse embryos, *Annals of West University of Timişoara, ser. Biology*, 5-6: 87-94

- Checiu I., Checiu M., Tuduce I., Ilut I., Hutanu D. 2008. Teratogenic effects of copper upon early postimplantational mouse embryos – in vitro experimental investigation, *Annals of West University of Timişoara, ser. Biology*, 11: 51-56
- Checiu M. Lupea A., Checiu Huţanu D., Checiu I. 2006.The effect of ethanol in vitro on preimplantation mouse embryos, *Annals of West University of Timişoara, ser. Biology*, 9: 43-55
- Checiu M. Lupea A., Checiu Huţanu D., Checiu I. 2007. In vitro effects of retinol upon developmental rate and viability of preimplantation mouse embryos, *Annals of West University of Timişoara, ser. Biology*, 10: 85-91
- Faur A, Ianovici N., Nechifor C. 2001. Airpalynology research implications in allergic diseases, *Annals of West University of Timişoara, ser. Biology*, 3-4: 7-14
- Filimon M. N. 2012. *Ecofiziologie animală şi umană*, Editura Mirton, Timişoara
- Filimon M. N., Sinitean A., Ianovici N., Borozan A., Bordean D. M., Dumitrescu G., Popescu R.. 2012. The influence of deoxynivalenol (DON) on hemoleucogram components at rats**,** *Analele Universităţii din Oradea - Fascicula Biologie*, XIX (1): 23-28
- Hou Y, Ding X, Hou W, Song B, Wang T, Wang F, Zhong J. Immunostimulant activity of a novel polysaccharide isolated from *Lactarius deliciosus*. *Indian J Pharm Sci* 2013;75:393-399
- Huţanu D. 2011. Experimental investigations reagarding the effects of bisphenol a in adult mice spermatogenesis, *Annals of RSCB*, 16 (2): 74-78
- Ianovici N. 2007. The principal airborne and allergenic pollen species in Timişoara, *Annals of West University of Timişoara, ser. Biology*, 10: 11-26
- Ianovici N. 2008. Aerobiological monitoring of allergenic flora in Timisoara, *Lucrări ştiinţifice. Seria Horticultură*, 51: 131-136
- Ianovici N., Dumbravă-Dodoacă M., Filimon M. N., Sinitean A. 2011. A comparative aeromycological study of the incidence of allergenic spores in outdoor environment, *Analele Universităţii din Oradea, Fascicula Biologie*, XVIII: 88-98
- Ianovici N., Maria C., Răduţoiu M.N., Haniş A., Tudorică D. 2013. Variation in airborne fungal spore concentrations in four different microclimate regions in Romania, *Not Bot Horti Agrobo*, 41(2):450-457
- Ianovici N., Ţărău G., Todosi A. L., Iriza E., Danciu A., Ţolea L., Tudosie D., Munteanu F., Bogdan D., Ciobănică V.. 2010. Contributions to the characterization of *Plantago* species from Romania. Review, *Annals of West University of Timişoara, ser. Biology*, 13: 37-76,
- Lee T.T., Huang C.C., Shieh X.H., Chen C.L. , Chen L.J., Yu B. 2012 Flavonoid, Phenol and Polysaccharide Contents of Echinacea Purpurea L. and Its Immunostimulant Capacity In Vitro, *International Journal of Environmental Science and Development*, 1 (1): 5-9
- Radhika P., Annapurna A., NageswaraRao S. 2012, Immunostimulant, cerebroprotective & nootropic activities of Andrographis paniculata leaves extract in normal & type 2 diabetic rats, *Indian J Med Res*,135, 636-641
- Ragap H.M., Khalil R.H., Mutawie H.H. 2012. Immunostimulant effects of dietary *Spirulina platensis* on tilapia *Oreochromis niloticus*, *Journal of Applied Pharmaceutical Science.* 2: 26-31
  Sahu M. S., Sahu R.A., Verma A. 2013. Immunomodulatory activity of alcoholic extract of Habenaria intermedia in mice, *International Journal of Pharmacy and Pharmaceutical Sciences*, 5, Suppl 3, 406-409
- Ramberg J.E, Nelson E.D., Sinnott R.A. 2010. Immunomodulatory dietary polysaccharides: a systematic review of the literature, *Nutrition Journal*, 9 (1): 2-22
- Simon D., Wardlaw A., Rothenberg M. E. 2010. Organ-specific eosinophilic disorders of the skin, lung and gastrointestinal tract, *J Allergy Clin Immunol*. 126 (1): 3–13.
- Tirziu E., Rugea T., Nichita I., Cumpănăsoiu C., Gros R.V., Seres M., 2009. Immunostimulant effect of fungal polysaccharides, Upon humoral immune response in broiler chickens, *Lucrări Stiinlifice Medicină Veterinară*, XLII (1), 340-345
- Van Gramberg J.L., de Veer M.J., O'Hehir R.E., Meeusen E. N. T., Bischof R.J. 2013. Use of Animal Models to Investigate Major Allergens Associated with Food Allergy, *Journal of Allergy*, 1-10
- Yu B., Lee T. T. 2010. Immunostimulant activity of *Echinacea* extracts *in vitro, Proceedings of 2010 International Conference on Biotechnology and Food Science* (ICBFS 2010) Bangalore, India, 9-10 February, 37-41
- Zheng W.F., Zhao Y.X., Zhang M.M., Yin Z.J., Chen C.F., Wei Z.W. 2008. Phenolic compounds from *Inonotus obliquus* and their immune-stimulating effects, *Mycosystema*. 27(4): 574-581