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Effect of *Pleurotus* spp. on the basic immunological parameters in mice

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Oral administration of 15 species of *Pleurotus* to mice (1 ml of water suspension/mouse) for two weeks showed marked increase in the peritoneal macrophages, total leucocytes, granulocytes-agranulocyte ratio and also haemoglobin content. This increase in the proliferation of macrophages, leucocytes etc. may be one of the mechanisms of mushroom induced immunostimulation.

Key words : Oyster mushrooms, *Pleurotus* sp., immunomodulatory properties, haemoglobin, peritoneal macrophages, leucocytes, granulocytes, agranulocytes

INTRODUCTION

Pleurotus species (Oyster mushrooms) enjoy a world — wide distribution in nature. They occur mostly as saprophytes on dead trunks of dead trees. They are distributed in both temperate and tropical zones ; however, they are more prevalent in the subtropics. *Pleurotus* species are now widely consumed as food in the East and increasingly in the West. Besides the ease of cultivation, the other reasons for its popularity during the last 15 years is its good nutritional value. The oyster mushrooms are good sources of non-starchy carbohydrates, have a high content of dietary fiber, contain moderate quantities of good quality protein with most of the essential amino acids, minerals, and vitamins (Bano and Rajarathanma, 1988 ; Breene, 1990 ; Opletal, 1993 ; Stamets, 1993). *Pleurotus* species have been shown to modulate the immune system. They have hypoglycemic activity and anti thrombotic effect, lower blood pressure and blood lipid concentration, and inhibit tumor growth, inflammation and microbial action. (Eisenhut and Fritz, 1991; Chang, 1993, 1996). According to *Pharmacopoea Sinica*, the medicinal part of the oyster mushrooms is its fruiting body — ''mild in nature and tasting sweet, it dispels air and cold and eases tendon and veins'' (Liu and Yun-Sun, 1980). According to Eastern folklore, *Pleurotus* can also prevent high blood pressure and artherosclerosis, impart long life and vigor, and assist people in recovering from fatigue. It can also prevent hangovers, constipation and improve sexual powers (Breene, 1990). The medicinal properties of *P. ostreatoroseus, P. smithii* and *P. ostreatus* reported from these countries are very similar to those described in Asia. Oyster mushrooms are supposed to be efficient as blood pressure lowering, diuretic, cholesterol reducing, adjuvant, and aphrodisiac agent (Guzman, 1994). In the present study an attempt was made to assess the basic immunological properties of selected species of the genus *Pleurotus*.

MATERIALS AND METHODS

Biological Material

The biological materials used for the present investigation are 15 species of basidiomycetes fungus coming under the order Agaricales, family Lentinaceae and genus *Pleurotus*. They are commonly called as 'Oyster mushrooms'. All are edible and widely cultivated world over (Table 1). Mycelial mats, grown on potato dextrese broth, were used for different analysis undertaken in the study.

Table 1 : Name and source of materials used for the investigation

Name	Source	Ref. No.
Pleurotus citrinopileatus	Microbial Type Culture Collections (MTCC), Chandigarh, India	1796
Pleurotus cystidiosus	Microbial Type Culture Collections (MTCC), Chandigarh, India	1797
Pleurotus eryngii	Microbial Type Culture Collections (MTCC), Chandigarh, India	1798
Pleurotus flabellatus	Microbial Type Culture Collections (MTCC), Chandigarh, India	1799
Pleurotus fossulatus	Microbial Type Culture Collections (MTCC), Chandigarh, India	1800
Pleurotus ostreatus	Microbial Type Culture Collections (MTCC), Chandigarh, India	1801
Pleurotus pulmonarius	Microbial Type Culture Collections (MTCC), Chandigarh, India	1805
Pleurotus sajor-caju	Microbial Type Culture Collections (MTCC), Chandigarh, India	1806
Pleurotus sapidus	Microbial Type Culture Collections (MTCC), Chandigarh, India	1807
Pleurotus membranaceus	National Centre for Mushroom Research, Solan, India	PL-90
Pleurotus platypus	National Centre for Mushroom Research, Solan, India	PL-180
Pleurotus florida	Regional Research Laboratory, Jammu, India	—
Pleurotus opuntiae	Regional Research Laboratory, Jammu, India	—
Pleurotus djamour	Regional Research Laboratory, Jammu, India	—
Pleurotus eous	Tamil Nadu Agricultural University, Coimbatore, India	Apk-1

Animals

Adult male Swiss Albino Mice (25-30 g) were used. They were fed with standard rodent pellet (Lipton & Co, Bangalore) and water *adlibitum*. They were bred and maintained at Tropical Botanic Garden and Research Institute's animal house under standard laboratory contitions.

Preparation of extracts

The mushroom mycelial mat of the fifteen *Pleurotus* species studied were harvested from potato dextrose broth cultures, rinsed repeatedly with distilled water. Moisture was removed by absorption on filter paper, and then dried at room temperature in the laboratory. The air-dried samples were powdered and the powder was weighed and ground into a paste. A 10% (w/v) suspension of the mycelial powder was prepared in water containing 2% gum acacia (GA). This aqueous suspension of the mycelial mat was administered orally to mice with the help of a canula (feeding tube) at a doze of 1.0 and / or 0.5 g of dried mycelial powder per kg of body weight.

Immunological studies

Mice were divided into two groups of eight animals each. Experimental groups received a daily oral dose of 0.5 ml suspension for two week. The control group received 2% gum acacia of appropriate volume.

Estimation of haemoglobin (Acid Haematin method)

Haemoglobin content was measured using haemoglobinometer with permanent coloured glass and comparison standards. Value was expressed as g/100 ml blood. In this estimation procedure, haemoglobin gets converted to acid heamatin by the action of hydrochloric acid.

Total Leucocyte count (TC)

Haemocytometer method was adopted for calculating the total leucocyte count. Blood was diluted with WBC diluting fluid, which removes the red cells by haemolysis and also accentuates the nuclei of the white cells. Counting was done with a microscope (low power, 100 × magnification) and knowing the volume of the fluid examined and dilution of the blood, the number of white cells per cu mm in undiluted whole blood was calculated.

Collection of peritoneal macrophages

Collection and counting of the peritoneal macrophages was done following the method described by Hudson and Hay (1980).

Differential count of leucocytes (Granulocyte — Agranulocyte ratio)

Leishman stain method was followed for determining the differential count. Smear was prepared from blood sample and approximately 100 leucocytes were counted and from that the ratio of granulocytes to that of agranulocytes was calculated

because these two populations of leucocytes differ in their functions.

RESULTS

Hemoglobin

Treatment (1ml of 2% suspension/mouse) for seven consecutive days, showed no marked difference in the blood hemoglobin content. Out of 15 *Pleurotus* species studied 12 species did not influence the blood hemoglobin content significantly. However, treatment with three species (*P. eryngii, P. sajor-caju* and *P. sapidus*) showed marked increase (Table 2).

Total leucocyte count

It was noticed that treatment (1ml of 2% suspension/mouse) for seven consecutive days, significantly influenced the leucocyte count in the blood of the treated animals. Except for a few species (*P. citrinopileatus, P. opuntiae* and *P. eous*) all others significantly enhanced the leucocyte count in the treated animals. (Table 2).

Peritoneal macrophages

Oral administration (1ml of 2% suspension/mouse) of all the 15 *Pleurotus* species resulted in dramatic increase in the number of peritoneal macrophages (Table 2). The highest count was noticed in animals treated with *Pleurotus fossulatus* (27.68 ± 0.48) and the lowest count with *P. cystidiosus* (12.81 ± 0.37).

Granulocyte-Agranulocyte ratio

Oral administration (1ml of 2% suspension/mouse) of almost all the *Pleurotus* species had numercially affected the granulocyte-agranulocyte ratio in mice (Table 2).

DISCUSSION

The observed increase in the leucocytes, peritoneal macrophages and granulocytes in almost all species indicate an increase in the immunomodulatory properties of the treated animals (Table 2). This suggests that all the *Pleurotus* species studied, directly or indirectly stimulate proliferation of macrophages, leucocytes etc., This could be one of the mechanisms of mushroom induced immunostimulation.

From the present investigation, it is clear that oral administration of different *Pleurotus* species enchances the immune function by way of affecting the cells responsible for the stimulation of the immune system (Table 2). The immunomodulatory properties in mushrooms are mainly due to the

Table 2 : Pharmacological evaluations

Name	Haemoglobin (g/100ml)	Total Leucocyte Count (Cells/ml × 10^{-6})	No. of Peritonial Macrophages (Cells/mouse × 10^{-6})	Granulocyte : Agranulocyte Ratio
Pleurotus citrinopileatus	16.01 ± 0.57	12.66 ± 0.43	13.08 ± 0.16	65 : 35
Pleurotus cystidiosus	16.68 ± 0.49	13.81 ± 0.38	12.81 ± 0.37	58 : 42
Pleurotus eryngii	20.12 ± 0.16	16.01 ± 0.41	18.31 ± 0.97	62 : 38
Pleurotus flabellatus	17.20 ± 0.28	15.11 ± 1.21	20.64 ± 0.16	56 : 44
Pleurotus fossulatus	18.07 ± 0.20	15.00 ± 0.89	27.68 ± 0.48	53 : 47
Pleurotus ostreatus	18.78 ± 0.24	14.08 ± 0.58	19.09 ± 0.16	68 : 32
Pleurotus pulmonarius	17.60 ± 0.46	15.61 ± 0.90	21.59 ± 0.57	51 : 49
Pleurotus sajor-caju	20.31 ± 0.31	15.98 ± 0.67	26.13 ± 0.08	55 : 45
Pleurotus sapidus	19.98 ± 0.36	14.81 ± 0.54	25.98 ± 0.32	63 : 37
Pleurotus membranaceus	18.67 ± 0.59	16.66 ± 0.35	12.96 ± 1.12	61 : 39
Pleurotus platypus	19.00 ± 0.49	14.97 ± 1.10	19.01 ± 0.69	66 : 34
Pleurotus florida	18.90 ± 1.11	15.67 ± 0.98	20.36 ± 0.74	58 : 42
Pleurotus opuntiae	16.01 ± 0.25	10.68 ± 0.54	23.09 ± 1.22	60 : 40
Pleurotus djamour	17.81 ± 0.81	14.93 ± 0.43	24.13 ± 0.28	56 : 44
Pleurotus eous	16.01 ± 0.01	11.71 ± 0.43	17.81 ± 0.41	58 : 42

Control : Haemoglobin — 14.30 ± 0.36 : Leucocytes — 8.16 ± 0.67 : Macrophages — 7.09 ± 1.12 ; Granulocyte ratio — 52 : 48
Values are mean ± S.D of 6 animals

presence of polysaccharides and they belong to 1-3-β-D-glucans and they have essentially the same structural features. In the present investigation, the component(s) reponsible for the numerical enhancement of certain cells may be due to the presence of these glucans. Glucans isolated from various *Pleurotus* species, appear to be prospective immunomodulative substances (Chihara et al., 1982 ; Jong et al., 1991 ; Chihara, 1993 ; Sakagami and Takeda, 1993 ; Mizuno, 1995). They stimulate humoral and cell mediated immunity, mechanism of non specific immunity and hemopoiesis. According to Gunde-Cimerman and Cimerman (1995) the immunological effects of glucan in mice are mainfested from increased protection from viral, bacterial, fungal and parasitic infections.

The increase in the peritoneal macrophages noticed in the present investigation in an important observation as they have crucial roles in the mechanism of immune stimulation. It is reported that macrophages play an important role in tumor cell lysis and tumor growth inhibition. They can be activated to tumorocidal state by a variety of agents like r-interferon, IL-2, etc.

The numerical increase in the granulocyte-agranulocytes ratios in the treated animals is also an indication of the immune stimulation. The leucocytes are the mobile units of the body's defense system. They are formed partly from the bone marrow (granulocytes) and partly from the lymph tissue (agranulocytes or lymphocytes and plasma cells). Defense system operates via granulocytes by phagocytosis and through agranulocytes by immunoglobulins. In most of the mammals, the granulocytes (with short life span of 4-8 will be always higher in number, i.e. almost 60% of the leucocytes. Lymphocytes having life span of months or even years will be 40% of the leucocytes. Any drug, which affects the numerical rartio of these cells, can be considered having potent effect on the immune system, which can either positively or negatively modulate the immune system. Assays giving the functional aspects of these cells only can tell the clear picture.

In inate immunity, the phagocytosis of foreign materials by macrophages and other phagocytes facilities the effect of function of these cells in homeostasis, host defense and inflammation. In acquired immunity, macrophages and other phagocytes contribute to regulation of both humoral and cellular immune responses. It is reported by several workers that generally mushrooms enhance both cell mediated and humoral immunity (Jong et al., 1991 ; Chihara, 1993 ; Mizuni, 1995). The observed anti-tumor effect of several mushrooms could be mediated by the enhancement of immune function.

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REFERENCES

Bano, Z. and Rajarathanam, S. 1988. Pleurotus mushrooms. Part II. Chemical composition, nutritional value, post harvest physiology, preservation and role as human food. *Crit Rev. Food Sci. Nutr.*, **27** : 87-158.

Breene, W. M. 1990. Nutritional and medicinal value of speciality mushrooms. *J. Food Protection.*, **53** : 883-894.

Chang, S. T. 1993. Mushroom biology : The impact on mushroom production and mushroom products. Keynote lecture. Ist *Intl. Conf. on Mush Biol. and Mushroom products*, 23-26, August, Hong Kong.

Chang, S. T. 1996. Mashroom research and development—equality and mutual benefits. In : *MUshroom biology and mushroom products*. (Royse, D. J., ed). Pensylvania State University, Univ. Park., pp. 1-10.

Chihara, G. ; Maeda, Y. Y. and Hamuro, J. 1982. Current status and prespectives of immunomodulators of microbial origin. *Int. J. Tissue React.*, **4** : 207-225.

Chihara, G. 1993. Medical aspects of Lentinan isolated from *Lentinus edodes* (Berk.) Sing. In : *Mushroom Biology and mushroom products*, Chang S. T., Buswell J. A. and Chiu S. W., eds, Chinese University Press, Hong Kong, 261-266.

Eisenhut, R. and Fritz, D. 1991. Medicinally effective and health promoting compounds of deible mushrooms. *Gartenbranuwissenschaft*, **56** : 266-270.

Gunde-Cimerman, N. and Cimerman, A. 1995. Pleurotus fruiting bodies contain an inhibitor of 3-hydroxy-3-methlyglutary-Coenzyme A reductase-lovastain. *Exper. Mycol.*, **19** : 1-6.

Guzman, G. 1994. The fungi in the traditional medicine in Meso-America and Mexico. *Revis Iberoamericana Micol.*, **11** : 81-85.

Hudson, L. and Hay, F. C. 1980. *Practical Immunology*, Blackwell, Oxford/London/Boston.

Jong, S. C.; Birmingham, J. M. and Pai, S. H. 1991. Immunomodulatory substances of fungal origin. *J. Immunol. Immunopharmacol.*, **9** : 115-122.

Liu, B. and Yun-Sun, B. 1980. *Fungi Pharmacopocia* (sinica). Kinoka Company, Oakland, CA. Pp. 297.

Mizuno, T. (ed). 1995. The versatile fungus-food and medicinal properties-chemistry, biochemistry, biotechnology and utilisation. *Food Rev. Intl.*, **11**, Marcel Dekker, New York, Basel, Hong Kong. P. 236.

Opletal, I. 1993. Phytotherapeutic aspects of diseases of the circulatory system, 2. The oyster mushrooms and its potential use. Cheskoslovenska Farmacia, **42** : 160-166.

Sakagami, H. and Takeda, M. 1993. Diverse biological activity of PSK (krestin), a protein-bound poly saccharide from *Coriolus versicolor* (Fr) Quel. In : *Mushroom Biology and Mushroo products*, eds. Chang ST, Buswell JA and Chiu SW. pp. 237-245. Hong Kong, Chinese University Press.

Stamets, P. 1993. *Growing Groumets and medicinal mushrooms.* Ten Speed Press, Berkely, CA. Pp 551.

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