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THE INFLUENCE OF IMMUNOSTIM PLUS – A STANDARDIZED FIXED COMBINATION OF *SCHIZANDRA CHINENSIS* WITH *ELEUTHEROCOCCUS SENTICOSUS* EXTRACTS ON BLOOD PHAGOCYTES AND LYSOZYME ACTIVITY IN PIGS

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The *in vivo* influence of radix *Eleutherococcus* and fructus *Schizandrae* combined extracts on blood phagocytes and lysozyme activity in females of pigs was studied. Feeding pig females for 7 days with Immunostim Plus (combined extract), 300 mg per day, increased the respiratory burst of blood phagocytes measured by colorimetric assay (RBA) and potential killing activity of blood phagocytes determined by colorimetric assay (PKA). Also the Immunostim Plus increased the lysozyme activity in serum measured by turbidimetric assay.

INTRODUCTION

Application of natural immunomodulatory products is one of the most important ways of preventing infectious diseases in humans and animals. *Eleutherococcus senticosus* is a commonly used herbal preparation with adaptogenic, anti-stress, and immuno-modulatory properties [Bohn *et al.*, 1987; Drozd *et al.*, 2002; Schmolz *et al.*, 2001; Steinmann *et al.*, 2001; Szołomicki *et al.*, 2000].

Schizandrae chinensis is largely used in China and has a long history of medical use as adaptogen and anti-oxidant due to its hepato-protective properties. It has also been prescribed in cases of chronic cough and dyspnea, diarrhea, night sweats, wasting disorders, irritability, palpitations and insomnia [Sinclair, 1998]. Decoctions of *Schizandrae* were found to possess strong anti-bacterial and anti-viral activities. Data on the immunotropic effects of *Schizandrae* is scarce but its anti-inflamatory and anti-tumor activities were described by many authors [Amaryan *et al.*, 2003; Hancke *et al.*, 1999; Hsieh *et al.*, 2002; Jung *et al.*, 1997; Lee *et al.*, 2003].

The aim of this study was to evaluate the combined influence of these two medical plant extracts (Immunostim Plus) on phagocytic ability and potential killing activity of blood phagocytes and lysozyme activity in serum of pigs.

MATERIAL AND METHODS

The study was performed on 10 WBP/PBZ females of pigs, weighing 45-50 kg, delivered from a farm cooperating with the Department of Veterinary Prophylaxis and Feed Hygiene, University of Warmia and Mazury in Olsztyn. The experimental material was the Immunostim Plus preparation (Herbapol Lublin), 300-mg capsules, composed of dried extract of *Eleutherococcus senticosus* radix (155 mg), dried extract of *Schizandra chinensis fructus* (100 mg), and adjunctive substances (50 mg). For 7 days the sows were administered (in the morning) with Immunostim Plus in daily doses of 300 mg (1 capsule per day). Blood for immunological study was separated from 10 pigs before and after oral application of Immunostim Plus (on day 8).

Leucocytes were isolated from blood by centrifugation at 2 000 g for 30 min at 4°C on the Gradisol G gradient (Aqua Medica, Poland), washed three time in PBS and resuspended in RPMI 1640 medium (Sigma, USA) supplemented with 10% of Foetal Calf Serum (FCS, Gibco-BRL) at a stock concentration of 2×10^6 cells/mL of medium.

The metabolic activity of blood phagocyting cells (mostly granulocytes) was determined on the measurment of intracellular respiratory burst after stimulation by PMA (phorbol myristate acetate, Sigma, USA), as described by Chung and Secombes [1988]. The isolated cells were resuspended in RPMI-1640 medium (Sigma, USA) at 10^6 cells/mL. On 96-well U-shaped microplates 100 μL of isolated blood phagocytes were mixed with of 100 μL of 0.2% nitro blue tetrazolium (NBT, Sigma, USA) solution in 0.2 mol/L phosphate buffer at pH 7.2 and supplemented with 1 μL of PMA at a concentration of 1 mg/mL of ethanol. After 30 min of incubation at 37°C, the supernatant was removed from each well. The cell pellet was washed with absolute ethanol and then three times in 70% ethanol and dried at room temperature. The amount of extracted reduced NBT after incuba-

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tion with 2 mol/L KOH and DMSO (dimethylsulfoxide, Sigma, USA) was measured at 620 nm in a plate microreader (MRX 3 Dynatech). All samples were tested in triplicate and the mean value served as the results.

Potential bactericidal activity of phagocytic cells was determined in isolated blood phagocytes stimulated with killed microorganisms according to the method presented by Rook *et al.* [1995]. To stimulate phagocytes, the killed *Staphylococcus aureus* strain 209 P was added at a concentration of $1\times10^6/100\ \mu$L. The blood phagocytes with killed bacteria were incubated for 1 h at 37°C and supernatant was decanted. The cell pellet was washed with absolute ethanol and next three times with 70% ethanol and dried at room temperature. This was followed by the addition of 2 mol/L KOH and DMSO to each well. The amount of extracted reduced NBT was measured at 620 nm in a plate microreader (MRX 3 Dynatech). All samples were tested in triplicate and the mean value served as the results.

Lysozyme activity in serum was measured by turbidimetric assay, according to the method presented by Anderson and Siwicki [1994].

For statistical analysis, means and standard deviations for all test values were calculated and Student's *t*-test was used to determine differences between two results. Differences were found significant at $p<0.05$.

RESULTS AND DISCUSSION

In the first experimental study, one week after oral application of Immunostim Plus, the immunostimulatory effects were observed in the examined pig females. The influence of Immunostim Plus on the phagocytic ability (RBA) and potential killing (PKA) activity of blood phagocytes is presented in Table 1. Feeding pigs Immunostim Plus highly significantly ($p<0.05$) increased the activities of phagocytes evaluated with all methods applied. On the other hand, Immunostim Plus administered for 7 days was found to statistically significantly ($p<0.05$) increase lysozyme activity in serum (Table 1).

In our preliminary experimental study, for the first time, the combination of *Eleutherococcus senticosus* and *Schizandra chinensis* extracts behaved as a strong immunostimulator of non-specific cellular defense, dependening on the first line cells, phagocytes. Also the combination of *Eleutherococcus senticosus* and *Schizandra chinensis* extracts increased the non-specific humoral defense mechanisms presented by lysozyme activity in serum. The experimental *in vitro* studies implied that *Eleutherococcus senticosus* exert the immunomodulatory influence on human lymphocytes [Bohn *et al.*, 1987; Schmolz *et al.*, 2001]. Another authors showed the stimulatory influence of *Eleutherococcus senticosus* on the activity of granulocytes and monocytes [Rogala *et al.*, 2003; Szołomicki *et al.*, 2000; Wagner *et al.*, 1985; Wildfeuer & Mayerhofer, 1994]. However, there are no reports on the influence of *Schizandra chinensis* on the activity of phagocytes. Also there are no papers about the effect of *Schizandra chinensis* on non-specific humoral defense mechanism presented by lysozyme activity.

TABLE 1. The effect of Immunostim Plus on the metabolic (RBA test) and phagocytic (PKA test) activity of blood leukocytes and lysozyme activity in serum of pigs (n=20, mean ± SE).

	Before application of Immunostim Plus	After application of Immunostim Plus
Respiratory Burst Activity (RBA) of blood phagocytes (OD 620 nm)	0.25±0.05	0.34±0.03*
Potential Killing Activity (PKA) of blood phagocytes (OD 620 nm)	0.29±0.05	0.41±0.06*
Lysozyme activity in serum (μg/mL)	2.35±0.4	3.58±0.5*

* Statistically significant at $p\leq0.05$

In our present study, the combination of *Eleutherococcus* and *Schizandra* extracts had a stimulatory influence on blood phagocyte function and lysozyme activity in females of pigs. In our earlier study we evaluated the influence of Immunostim Plus on the activities of phagocytes and lymphocytes in mice [Siwicki *et al.*, in press; Skopińska-Różewska *et al.*, in press]. The results showed that Immunostim Plus stimulated the granulocytes and lymphocytes activity in mice. This study demonstrated also that Immu nostim Plus stimulated the graft-host activity of splenic lymphocytes and diminished neovascular response induced by transplanted cells in recipient mice [Siwicki *et al.*, in press]. This study sugested that Immunostim Plus may be applied during infections to improve non-specific cell-mediated and humoral-mediated immunity in response to pathogens.

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REFERENCES

1. Amaryan G., Astvatsatryan V., Gabrielyan E., Panossian A., Panosyan V., Wikman G., Double-blind placebo-controlled, randomized, pilot clinical trial of ImmunoGuard – a standarized fixed combination of *Andrographis paniculata* Nees, with *Eleuterococcus senticosus* Maxim, *Schizandra chinensis* Bail. and *Glycyrrhiza glabra* L. extracts in patients with familial Mediterranean Fever. Phytomedicine, 2003, 10, 271–285.
2. Anderson D.P., Siwicki A.K., Simplified assays for measuring nonspecific defense mechanisms in fish. Fish Health Section. Am. Fisheries Soc. Meeting, Seattle, WA, USA, 1994, pp. 1–26.
3. Bohn B., Nebe C.T., Birr C., Flow-cytometric studies with *Eleuterococcus senticosus* extract as an immunomodulatory agent. Arzneim-Forsch., 1987, 37, 1193–1196.
4. Chung S., Secombes S.J., Analysis of events occurring within teleost macrophages during the respiratory burst. Biochem. Physiol., 1988, 89B, 539–544.
5. Drozd J., Sawicka T., Prosinska J., Estimation of humoral activity of *Eleuterococcus senticosus*. Acta Pol. Pharm., 2002, 59, 395–401.
6. Hancke J.L., Burgos R.A., Ahumada F., *Schisandra chinensis* (Turcz). Baill. Fitoterapia, 1999, 70, 451–47.
7. Hsieh T.C., Lu X., Guo J., Xiong W., Kunicki J., Wu J.M., Effects of herbal preparation Equigard on hormone-responsive and hormone-refractory prostate carcinoma cells: mechanistic studies. Int. J. Oncol., 2002, 20, 681–689.

8. Jung K.Y., Lee I.S., Sir Oh., Kim D.S., Lee H-K., Lignans with plateled activating factor antagonist activity from *Schisandra chinensis* (Turch). Baill. Phytomedicine, 1997, 4, 229–231.
9. Lee I.S., Lee H-K., Dat N.T., Lee M.S., Kim J.W., Na D.S., Kim Y.H., Lignans with inhibitory activity against NFAT transcription from *Schisandra chinensis*. Planta Med., 2003, 69, 63–64.
10. Rogala E., Skopińska-Różewska E., Sawicka T., Sommer E., Prosinska J., Drozd J., The influence of *Eleuterococcus senticosus* on cellular and humoral immunological response of mice. Pol. J. Vet. Sci., 2003, 6, 37–39.
11. Rook G.A.W., Steele J., Umar, Dockrell H.M., A simple method for the solubilization of reduced NBT and its use as a colorimetric assay for activation of human macrophages by gamma interferon. J. Immunol. Meth., 1995, 82, 161–167.
12. Schmolz M.W., Sacher F., Aicher B., The synthesis of Rantes, G-CSF, IL-4, IL-5, IL-6, IL-12 and IL-13 in human whole-blood cultures is modulated by an extract from *Eleuterococcus senticosus* L. roots. Phytother. Res., 2001, 15, 268–270.
13. Sinclair S., Chinese Herbs: A Clinical Review of *Astragalus*, *Ligusticum* and *Schizandrae*. Altern. Med. Rev., 1998, 3, 338–344.
14. Siwicki A.K., Skopińska-Różewska E., Nartowska J., Małaczewska J., Wójcik R., Sommer E., Trapkowska S., Filewska M., Skurzak H., The effect of Immunostim Plus – a standarized fixed combination of *Schizandra chinensis* with *Eleuterococcus senticosus* extracts on granulocytes activity and tumour angiogenesis in mice. Bull. Vet. Inst. Pulawy – in press.
15. Skopińska-Różewska E., Siwicki A.K., Nartowska J., Małaczewska J., Wójcik R., Sommer E., Makula J., Skopiński P., Filewska M., Trapkowska S., Lewicka-Tarchalska M., Bany J., Immunostimulatory effect of Immunostim Plus – a standarized fixed combination of *Schizandra chinensis* with *Eleuterococcus senticosus* extracts on lymphocyte-dependent cellular immunity and immunological angiogenesis in mice. Acta Vet. Brno – in press.
16. Steinmann G.G., Esperester A., Joller P., Immunopharmacological *in vitro* effect of *Eleuterococcus senticosus* extracts. Arzneimittelforschung., 2001, 51, 76–83.
17. Szołomicki S., Samochowiec L., Wójcicki J., The influence of active components of *Eleuterococcus senticosus* on cellular defence and physical fitness in man. Phytother. Res., 2000, 14, 30–35.
18. Wagner H., Proksch A., Riess-Maurer I., Vollmar A., Odenthal S., Stuppner H., Jurcic K., Le Turdu M., Fang J.N., Immunostimulating action of polysaccharides (heteroglycans) from higher plants. Arzneimittelforschung., 1985, 35, 1069–1075.
19. Wildfeuer A., Mayerhofer D., The effects of plant preparations on cellular functions in body defense. Arzneimittelforschung., 1994, 44, 361–366.