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DOES GARLIC (ALIOFIL) INFLUENCE THE IMMUNE SYSTEM OF CHILDREN? – A PRELIMINARY STUDY

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According to the results of studies in humans and animals – which have shown some positive influence of garlic on their immune systems – we have undertaken a similar pilot investigation in children with recurrent infections of respiratory tract and mild cellular immunodeficiency. 15 children aged 3–15 years were given a preparation of dry garlic in tablets (Aliofil) for 10 days in 3 following months. Clinical and immunological investigations were carried out before and after the treatment. Statistically significant improvement of T lymphocytes function ($p=0.005$), evaluated by the blastic transformation method, as well as the improvement of children clinical condition were obtained. Other investigated immunological parameters remained unchanged.

INTRODUCTION

Garlic was known as a useful herb a long time ago. Egyptian, Korean and Chinese historical data indicated that it was used as a medicament in various disorders. Now, a great "come back" of this ancient herb can be noticed. A great influence of garlic on human health has been observed in clinical and experimental studies. These health-promoting properties of garlic have been the subject of many investigations and scientific reports, even in the last decade [Patya *et al.*, 2004; Kleczkowski *et al.*, 2004; Sela *et al.*, 2004; Siddique & Afzal, 2004; Chang *et al.*, 2004; Hassan *et al.*, 2003; Zhang *et al.*, 2002; Ghazanfari *et al.*, 2002; Hodge *et al.*, 2002; Colic *et al.*, 2002; Lutomski, 2001; Ge *et al.*, 1999; Tang *et al.*, 1997].

Traditional herbal medicines appear to constitute a rich source of active substances for immunotherapy [Hadden, 1993]. Garlic exhibits anti-infectious properties as well as exerts anticarcinogenic influence and prevents cardiovascular diseases. Immunologic and antioxidant activities of different types of garlic preparations have also been examined.

All the above-mentioned works, carried out *in vitro* as well as *in vivo*, in animals and humans have documented the activation of T lymphocytes, interleukin-2 biosynthesis, natural killer cells, polymorphonuclear leukocytes by garlic and its influence on monocytes and their vascular endothelial cell adhesion. Taking into account these promising scientific findings we have started our study in children.

The aim of our pilot investigations was to evaluate potential beneficial effects of garlic (Aliofil) on the immune system and health condition of children with recurrent infections of respiratory tract and mild cellular immunodeficiency.

MATERIALS AND METHODS

The study was carried out on the group of 35 children aged between 3 and 15 years: 25 children who were directed to the Immunology Outpatient Clinic of Mother and Child Institute due to recurrent respiratory inflammations and laboratory confirmed mild cellular immunodeficiency and 10 healthy children at the same age (waiting for small surgery correction). 25 children were qualified according to inclusion and exclusion criteria.

The criteria for the inclusion:

1. Age of children: 3-15 years, recurrent infections of respiratory tract, difficult to cure – in medical interview.
2. Laboratory confirmed defect of cellular immunity (decreased total number and/or percentage of lymphocytes T, defect of their function, phagocytosis defect).
3. No immunostimulators and corticosteroides given in period of 6 months before investigation.
4. No immunotherapy or vaccination performed in the time of 6 months before the investigation.
5. No antibiotics given in the last 2 months.

The criteria for the exclusion:

1. To fail in conditions of criteria for the inclusion in the points 1 to 5.
2. Renal insufficiency, gout, urolithiasis, hypersensitivity to the drug, actual infection.

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Children qualified for the treatment (based on the criteria of including and excluding) were given dry garlic in tablets (Aliofil) 2–3 tabl./daily (according to age) in divided doses for ten days in three following months. After the treatment, all children were observed for 12 months in the Immunology Outpatient Clinic.

The results of Aliofil treatment were estimated using special clinical scale as well as immunological laboratory evaluation. The clinical scale includes such parameters as the number of recurrent infections, degree of exacerbation, time and amount of cure with antibacterial drugs in the last 2 years before this treatment in comparison with 12 months after its discontinuation.

Immunological investigations in healthy children were carried out once. Their results were normal and used as additional actual, referential values. The results were statistically analysed using the T-test for paired sample comparison data in Statgraphics *Plus* Program ver. 2.1.

The following laboratory examinations were performed in 15 children (out of 25 clinically observed) before and after the treatment: (i) lymphocytes T – rosettes test; (ii) lymphocytes blast transformation test; (iii) cytometric analysis of peripheral blood lymphocytes subclasses using an EPICS XL flow cytometer by Beckmann Coulter; (iv) endocytosis of neutrophils (with latex); (v) NBT test; (vi) chemiluminescence of neutrophils; and (vi) IgG, IgA, IgM concentration in serum.

The details of the methods mentioned above have been described in a manuscript Gołębiowska-Wawrzyniak *et al.* [2004].

RESULTS AND DISCUSSION

The results of Aliofil influence on some parameters of children immune system in our study varied (Tables 1, 2, 3). The best stimulatory effect, with statistically highly significant difference (p=0.005), was obtained for the results of lymphocyte blast transformation test (LBTT) with PHA (Table 1).

In flow cytometric analysis (Table 2), the number of CD3 (lymphocytes T), CD4 (helper T lymphocytes), CD8 (cytotoxic T lymphocytes) and CD19 (B lymphocytes) showed the tendency to increase but without any statistical significance.

TABLE 1. Comparison of selected parameters of cellular immunity in children before and after the administration of Aliofil.

Number of patients n = 15		Before treatment		After treatment
Lymphocytes T - rosettes test- (cells /μL)	x	2606.9		2652.8
	p		0.72	
Lymphocytes blast transformation test with PHA (% of cells)	x	55.7		70.1
	p		0.005	
Endocytosis of neutrophils (% of cells)	x	99.4		99.2
	p		0.74	
NBT test (% of cells)	x	2.0		2.3
	p		0.31	
Chemiluminescence of neutrophils (index of stimulation)	x	4.1		4.3
	p		0.41	

TABLE 2. Comparison of selected lymphocyte's markers in children before and after the administration of Aliofil.

Number of patients n = 15		Before treatment		After treatment
CD3+ **T lymphocytes** (cells /μL)	x	1810		1905
	p		0.6	
CD4+ **helper T lymphocytes** (cells /μL)	x	935		1158
	p		0.19	
CD8+ **cytotoxic T lymphocytes** (cells /μL)	x	588		600
	p		94	
CD19+ **B lymphocytes** (cells /μL)	x	451		587
	p		0.25	
CD16+CD56+ **NK cells** (cells /μL)	x	392		246
	p		0.04	
CD3+/HLA-DR **active T lymphocytes** (cells /μL)	x	103		76
	p		0.08	
ratio CD4/CD8	x	1.8		2
	p		0.1	

Surprisingly, the number of CD16/CD56 (NK cells) and CD3/HLA-DR (active T lymphocytes) tended to decrease, which in the case of the former one was with the statistically significant difference. However, the function of these cells is more important but was not investigated.

No significant changes were obtained for values of endocytosis, NBT test, chemiluminescence of neutrophils as well as in serum concentrations of IgG, IgA and IgM (Tables 1 and 3).

TABLE 3. Changes of serum immunoglobulins concentration in children before and after the administration of Aliofil.

Number of patients n = 15		Before treatment		After treatment
IgG (IU/mL)	x	113.6		121.2
	p		0.22	
IgA (IU/mL)	x	74.8		79.1
	p		0.21	
IgM (IU/mL)	x	123.1		120.0
	p		0.50	

This indicates that the immunostimulative action of garlic may be connected first of all with its influence on the function of T lymphocytes. The same was also observed in other studies concerning T lymphocytes and such cells as NK cells and neutrophils [Patya *et al.*, 2004; Kleczkowski *et al.*, 2004; Sela *et al.*, 2004; Siddigue & Afzal, 2004; Chang *et al.*, 2004; Hassan *et al.*, 2003; Zhang *et al.*, 2002; Ghazanfari *et al.*, 2002; Hodge *et al.*, 2002; Colic *et al.*, 2002; Lutomski, 2001; Ge *et al.*, 1999; Tang *et al.*, 1997].

This improvement of cellular immunity induced by garlic and confirmed by our pilot study may be important for the prophylaxis of infections. The antimicrobial effects of garlic preparations are well established. Garlic (*Allium sativum*) demonstrates antiviral [Tatarintsev *et al.*, 1992;

Weber *et al.*, 1991], antibacterial [Chen *et al.*, 1999; Chung *et al.*, 1998], antimycotic [Pai & Platt, 1995; San-Blas *et al.*, 1989; Yoshida *et al.*, 1987; Tansey *et al.*, 1975], and even antiparasitic [Bany, 2003] properties.

The condition of the examined children was found to improve upon the administration of Aliofil. All main parameters describing the length, heaviness and scores of the illness were observed to decrease, namely:

- the number of recurrent respiratory infections per year decreased by 78.6% (equivalent to 4.68 times less infections than before treatment by Alliofil);
- average degree of exacerbation decreased by 49.2% (1.97 times less);
- the number of antibiotic treatments per year decreased by 85.7% (7.0 times less);
- the number of treatment with other non-antibiotic drugs decreased by 78.9% (4.74 times less);
- time length of the illness decreased by 85.4% (6.87 times less).
- and finally, the overall score index of clinical symptoms decreased by 67.7% (3.09 times less).

The results presented above are the clinical confirmation of the immunomodulatory possibilities of garlic. Kyo *et al.* [1998, 2001], on the basis of their works, strongly suggested that garlic (aged garlic extract – AGE) could be a promising candidate for an immune modifier, which maintains the homeostasis of immune functions. The same opinion was presented in the following publications: Colic & Savic [2000], Colic *et al.*, [2002], Ghazanfari *et al.*, [2002], Hassan *et al.*, [2003], Patya *et al.*, [2004], and Sela *et al.*, [2004].

It must be underlined that garlic is uniquely the richest dietary source of many otherwise rare health-promoting sulfur compounds and organic selenium, as well as one of the best sources of organic germanium, besides containing an impressive array of other essential nutrients and active health-promoting phytochemicals.

Many active substances such as allicin, the main organic allyl sulfur component, 14-kDa glycoprotein and others were obtained from garlic. Various forms of garlic are available, the most effective being fresh, powdered, distilled and especially aged garlic, which lacks the irritant effect of fresh garlic, yet possesses equal or greater bioactive range and potency.

In this study, children were administered with a dry garlic preparation – Aliofil.

In conclusion we would like to underline that our preliminary study brought some immunological as well as clinical data indicating a beneficial influence of garlic on children's health.

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