Phytomedicine 10: □□□–□□□, 2003

http://www.urbanfischer.de/journals/phytomed

Phytomedicine

Double-blind, placebo-controlled, randomized, pilot clinical trial of ImmunoGuard® – a standardized fixed combination of *Andrographis paniculata* Nees, with *Eleutherococcus senticosus* Maxim, *Schizandra chinensis* Bail. and *Glycyrrhiza glabra* L. extracts in patients with Familial Mediterranean Fever

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Summary

Double blind, randomized, placebo controlled pilot study of ImmunoGuard® – a standardized fixed combination of *Andrographis paniculata* Nees., *Eleutherococcus senticosus* Maxim., *Schizandra chinensis* Bail., and *Glycyrrhiza glabra* L. special extracts standardized for the content of Andrographolide (4 mg/tablet), Eleuteroside E, Schisandrins and Glycyrrhizin, was carried out in two parallel groups of patients. The study was conducted in 24 (3–15 years of both genders) patients with Familial Mediterranean Fever (FMF), 14 were treated with tablets of series A (verum) and 10 patients received series B product (placebo). The study medication was taken three times of four tablets daily for 1month. Daily dose of the andrographolide – 48 mg. The primary outcome measures in physician's evaluation were related to duration, frequency and severity of attacks in FMF patients (attacks characteristics score). The patient's self-evaluation was based mainly on symptoms – abdominal, chest pains, temperature, arthritis, myalgia, erysipelas-like erythema. All of 3 features (duration, frequency, severity of attacks) showed significant improvement in the verum group as compared with the placebo. In both clinical and self evaluation the severity of attacks was found to show the most significant improvement in the verum group. Both the clinical and laboratory results of the present phase II (pilot) clinical study suggest that ImmunoGuard® is a safe and efficacious herbal drug for the management of patients with FMF.

Key words: *Andrographis paniculata* fixed combination, ImmunoGuard®, Familial Mediterranean Fever, Placebo controlled parallel group clinical trials

Introduction

Familial Mediterranean fever (FMF), also called Periodic Disease or more rarely Recurrent Polyserositis, is a genetic disorder characterized by recurrent febrile episodes and inflammation of serous membranes. It is transmitted in an autosomal recessive form of inheritance and affects mainly ethnic groups living around the Mediterranean basin: Jews, Armenians, Turks, Arabs, Greeks, Druze. FMF is characterized by unpro-

0944-7113/03/10/0 - $ 15.00/0

voked intermittent attacks of fever with pain, affects the joints, pleural space and peritoneal cavity, its symptoms are not apparent between attacks. The inflammatory episodes begin in childhood, generally last 2–4 days, and resolve without sequels (Sohar et al. 1967).

The gene causing FMF, designated MEFV, is expressed in mature neutrophils, suggesting that it functions as an inflammatory regulator. Among peripheral blood leukocytes, MEFV expression was detected in neutrophils, eosinophils, and to varying degrees, monocytes. In vitro stimulation of monocytes with the pro-inflammatory agents interferon (IFN) gamma, tumor necrosis factor, and lipopolysaccharide induced MEFV expression, whereas the antiinflammatory cytokines interleukin (IL) 4, IL-10, and transforming growth factor beta inhibited such expression. In granulocytes, MEFV was up-regulated by IFN-gamma and the combination of IFN-alpha and colchicine, identifying it as an IFN-gamma immediate early gene (Centola et al. 2000). Cytokine transcriptional pathways are misregulated in attack-free FMF patients, that supports the hypothesis that these patients have subclinical inflammation between attacks (Notarnicola et al. 2002). An altered pattern of tumor necrosis factor (TNF) secretion in patients with familial Mediterranean fever (FMF) was demonstrated (Schattner et al. 1991; 1996; Dilsen et al. 1992). It was suggested that the cytokine network is activated during attacks of FMF and IL-6 appears to play an important role in the evolution of FMF attacks (Gang et al. 1999).

For today, Colchicine is the only remedy known to be consistently effective in preventing the acute attacks of familial Mediterranean fever (FMF) (Goldfinger 1972; Ozkan et al. 1972; Zemer et al. 1986). Colchicine has, however, a low therapeutic index; steady-state plasma concentration is 0.5–3 ng/ml, while toxicity appears at 3 ng/ml. Stages in serious Colchicine intoxication includes gastrointestinal disturbances, respiratory distress, cardiovascular collapse, paralytic ileus, rhabdomyolysis and renal failure, delirium and coma. Side effects of colchicines are mostly due to binding of colchicine to tubulin and in daily clinical practice are of gastrointestinal origin: nausea, vomiting, abdominal cramps and diarrhea. Colchicine induces, leukopenia, thrombocytopenia, myopathy, neuropathy, severe and proximal weakness. Seizures may occur. A large range of lethal doses has been reported. Another disadvantage of the Colchicintherapy is that it may interfere with the absorption of various drugs and vitamins by its effect on the intestinal mucosa. Colchicine metabolism in the liver utilizes the CYP 3A4 isoform of cytochrome P-450 system, there is the potential danger of interaction with numerous drugs (Tunca, 2000; Guven et al. 1999; Ben-Chetrit and Levy, 1998).

Since colchicine still remains the only available treatment of FMF, the search of new non-toxic anti-inflammatory drugs, which can replace Colchicine in FMF is of practical importance.

It is known that corticosteroids and conventional nonsteroid antiinflammatory drugs (NSAID) are not effective in FMF. Diterpene lactone andrographolide, an active principle of *Andrographis paniculata* Nees used for prevention and treatment of common cold in Scandinavia (Melchior et al. 1997; Caceres et al. 1997). is known as an antiinflammatory and analgesic agent (Madav et al. 1996; Madav et al. 1995; Gupta et al. 1998; Singh and All, 1994) The mechanism of its action is not connected with inhibition of the biosynthesis of eicosanoids, as in conventional non-steroidal anti-inflammatory drugs (NSAID) mode of action (Amroyan et al. 1999). It was demonstrated that anti-inflammatory effects of *Andrographis paniculata* is likely associated with inhibition of PAF-mediated inflammatory response (Amroyan at al., 1999), inhibition of expression of nitric oxide (NO) synthesis in macrophages (Chiou et al. 1998), and modulation of cytokines (INF-γ, TNF-α) production (Panossian et al. in press 2002). Unique mode of anti-inflammatory action of Andrographis, its use in traditional systems of medicine (Chang and But, 1986; *Standard of ASEAN herbal medicine*, 1993; *Pharmacopoeia of the People's Republic of China*, 1992; Tang and Eisenbrand, 1992; Farnsworth, NF ed. NAPRALET Database, 1998), supported by clinical studies of Andrographis preparations in patients with upper respiratory infections such us common cold, sinusitis, bronchitis, pharyngotonsillitis, lower urinary tract infections and acute diarrhea (Melchior et al. 1997; Caceres et al. 1997; Thamlikitkul et al. 1991; Chaichantipyuth and Thanagkul, 1986; Thanagkul and Chaichantipayut, 1985; Chaturvedi, 1983; Burkill, 1966; Singh and All, 1994) as well as a recent findings indicating that interferon alpha (IFN) may ameliorate acute FMF attacks and substantially reduced the magnitude of the acute phase response during attacks of FMF (Tunca et al. 2000), let us assume that it might be beneficial in the treatment of FMF.

In this study we evaluated clinical efficacy of a standardized *Andrographis paniculata* Herba Nees extract fixed combination with ImmunoGuard® in FMF. General aim of this placebo controlled clinical trial was evaluation of the efficiency of ImmunoGuard® tablets as a prophylactic and curative agent in FMF using randomized selection of patients. For this purpose effect of ImmunoGuard® on the development of the serosal inflammation (acute attacks of FMF), duration, severity, frequency of these attacks as well as on body temperature, abdominal and chest pains, arthropathy, myalgia, erysipelas-like erythema in children suffering from FMF.

Subjects, Materials and Methods

Study Drug

The test medication (verum and placebo) was manufactured according to Good Manufacturing Practice (GMP) by Swedish Herbal Institute (SHI) in the form of white, sugar-coated tablets.

• *Verum tablets*: ImmunoGuard® Clinical A, 370 mg, containing a fixed combination of *Andrographis paniculata* Nees special extract (50 mg) standardized for the content of andrographolide (3-[2-[-decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene 1-naphthalenyl] ethyldiene] dihydro-4-hydroxy-2(3H)-furanone) – 4 mg, *Eleutherococcus senticosus* special extract (10 mg) standardized for the content of Eleutherosid E (>0.8 mg), *Schizandra chinensis* special extract (100 mg) standardized for the content of Schisandrins (>0.8 mg), *Glycyrrhiza glabra* L extract (10 mg) standardized for the content of Glycyrrhizin (>0.6 mg), 190.3 mg micro crystalloid cellulose, 7.4 mg Syloid FP, 1.8 mg magnesium stearate, 0.8 mg shellac, 0.1 mg olive oil, and 0.07 mg Macrogol.

• *Placebo Tablets*: ImmunoGuard® Clinical B. containing lactose 170 mg, calcii phosphas dibasicus, solani amylum, cellulosum microcristallinum, magnesii stearas, silica colloidalis anh.

• *Coating for both verum/placebo:* Saccharose, calcium carbohydrate, magnesium silicate, polyvinylpyrrolidone, titan dioxide.

All tablets were coated, had similar appearance and package so that the placebo and drug could not be distinguished from each other. They were kept under safe conditions (in a special room) at room temperature with an order not to be used for any other purposes but the study. Each package of tablets contained 60 tablets to be taken 4 × 3 tablets daily for 5 days. An identification number was noted in a protocol to allow a subsequent identification after the completion of study and performed statistical analysis.

The information on placebo and the active substance became available to the investigators and volunteers only after the completion of study and after the statistical analysis was performed.

The study was performed with the revised declaration of Helsinki (World Medical Association Declaration of Helsinki – 1964, 2000). The protocols of the study were reviewed and approved by Ethic Committee of Armenian Drug and Medical Technology Agency of the Ministry of Health of the Republic of Armenia.

Analytical methods

Validated methods of analysis of andrographolide, schisandrin (supplied by Swedish Herbal Institute, Sweden) eleutherosides B and E (generous gift of G. Zapesochnaya, VILAR, Russia) and glycyrrhizin (Extrasynthese, France) in tablets by HPLC were used. Quantitation of andrographolide, schisandrin and glycyrrhizin was performed by internal standard (Ethyl 4-hydroxybenzoate, Aldrich) method (A), Eleuterosides E and B – by external standards method (B).

• *Instrumentation*

Beckman HPLC "GOLD" system consisting of:

Detector:	BECKMAN UV-Detector Module 166
Pump:	BECKMAN Double pump Programmable Solvent Module 125
Injection Valve:	Rheodyne mod. 7725I with 20 µl loop.
Data Collection:	PS/1 Computer 486 DX-33 with management software supplied by Beckman; Epson FX-800 printer.
Statistical analysis**:**	Prism software, version 2.0, GraphPad Software Inc. USA, 1996

Experimental conditions, method A- determination of andrographolide, schisandrin and glycyrrhizin

Column	*LiChroCART 125 × 4 mm HPLC cartridge with LiChrospher100 RP-18 (5 µm) (Merck, Darmstadt)*
Mobile phase	*Pump A – Water-acetic acid (100:0.1, v/v);* *Pump B – Acetonitrile-acetic acid (100:0.1, v/v)*
Gradient	*Min.* — *%B*: *00* — *20*; *00–11* — *20–45*; *11–20* — *45–55*; *20–25* — *55–100*; *25–28* — *100–100*; *28–30* — *100–20*; *30* — *20*
Flow rate	*0.7ml/min*
Detection	*Min.* — *UV, nm*: *00–11.5* — *229 nm*; *11.5–30* — *250 nm*
Temperature	*Ambient (about 25 °C)*
Injection volume	*20 µl*

• *Calculations*

– *Andrographolide:*

$$m_{AND} = m_{IS} \cdot \frac{S_{AND}}{S_{IS}} \cdot \frac{1}{K_{AND}}$$

m_{AND} – content of the Andrographolide in ImmunoGuard®, (mg/tab)
m_{IS} – weight of internal standard in analyte solution (AS), mg (1.0 mg)
S_{AND} – area of Andrographolide peak in AS
S_{IS} – area of the internal standard peak in AS
K_{AND} – ratio of peaks areas of Andrographolide and internal standard in Standard Solution (SS),

$$K_{AND} = \frac{S_{AND} \cdot C_{IS}}{S_{IS} \cdot C_{AND}},$$

C_{AND} – concentration of the Andrographolide in SS, (0.1 mg/ml)
C_{IS} – concentration of the internal standard in SS, (0.05 mg/ml)
S_{AND} – area of Andrographolide peak in SS
S_{IS} – area of the internal standard peak in SS

– *Glycyrrhizin:*

$$m_{Gly} = m_{IS} \cdot \frac{S_{Gly}}{S_{IS}} \cdot \frac{1}{K_{GLY}}$$

m_{Gly} – content of the Glycyrrhizin in Immuno-Guard, (mg/tab)
m_{IS} – weight of internal standard in AS, mg (1.0 mg)
S_{Gly} – area of Glycyrrhizin peak in AS
S_{IS} – area of the internal standard peak in AS
K_{GLY} – ratio of peaks areas of Glycyrrhizin and internal standard in Standard Solution (SS),

$$K_{GLY} = \frac{S_{Gly} \cdot C_{IS}}{S_{IS} \cdot C_{Gly}},$$

C_{Gly} – concentration of the Glycyrrhizin in SS, (0.1 mg/ml)
C_{IS} – concentration of the internal standard in SS, (0.05 mg/ml)
S_{Gly} – area of Glycyrrhizin peak in SS
S_{IS} – area of the internal standard peak in SS

– *Schisandrin:*

$$m_{Sch} = m_{IS} \cdot \frac{S_{Sch}}{S_{IS}} \cdot \frac{1}{K_{SCH}}$$

m_{Sch} – content of the Schisandrin in Immuno-Guard, (mg/tab)
m_{IS} – weight of internal standard in AS, mg (1.0 mg)
S_{Sch} – area of Schisandrin peak in AS
S_{IS} – area of the internal standard peak in AS
K_{SCH} – ratio of peaks areas of Schisandrin and internal standard in Standard Solution (SS),

$$K_{SCH} = \frac{S_{Sch} \cdot C_{IS}}{S_{IS} \cdot C_{Sch}},$$

C_{Sch} – concentration of the Schisandrin in SS, (0.1 mg/ml)
CI_S – concentration of the internal standard in SS, (0.05 mg/ml)
S_{Sch} – area of Schisandrin peak in SS
S_{IS} – area of the internal standard peak in SS

• *Method B – Determination of Eleutherosides B and E*

Column: *LiChroCART 250–4 mm HPLC cartridge with Superspher60 RP-select B (Merck, Darmstadt)*

Mobile phase: *Pump A – Water*
Pump B – Acetonitrile

Gradient:

Min.	*%B*
00	*15*
00–16	*15–25*
16–20	*25–25*
20–25	*25–80*
25–28	*80–15*
28	*15*

Flow rate: 0.*7 ml/min*
Detection: *UV = 210 nm*
Temperature: *Ambient (about 25 °C)*
Injection volume: *20 µl*

Study Design

The current study is a double blind placebo-controlled trial. Modalities of the trial were explained to the parents of patients with FMF who volunteered for the trial. The parents were informed that ImmunoGuard® is an herbal product developed from medicinal plants by Swedish Herbal Institute. The summary of both the laboratory and clinical data accumulated up to date was explained to volunteers and their parents in simple non-technical language. The volunteers and their parents asked questions on areas of the presentation about which they needed further clarification. Furthermore, the patients and their parents were informed that they could withdraw at anytime during the trial while the clinical investigator could advise any volunteer to withdraw from the trial if he/she develops adverse reaction to ImmunoGuard®.

Each patient was provided 6 jars containing 360 tablets to be able take 4 tablets 3 times a day for 1 month. Placebo tablets were organoleptically the same to make them indistinguishable from the verum ImmunoGuard® clinical B.

All tablets were coated, had similar appearance and package so that the placebo and drug could not be distinguished from each other. They were kept and safe conditions (in a special room) at room temperature with an order not be used for any other purposed but the study.

Written informed consent was subsequently obtained from each parent or guardian of patients. The Armenian Drug and Medical Technologies Agency, and the Ethical Committee of ADMTA approved the study.

The study was carried out at Republican children's Center of FMF (Yerevan State Medical University clinic) in Republican Children Hospital during January 2001–January 2002.

The clinical investigation was carried out as a randomized parallel-group trial with a verum group and a placebo group. The trial was performed to investigate the efficacy and tolerability of ImmunoGuard® in children with Familial Mediterranean Fever. The evaluation was based on the patient's self-evaluation and the physician's evaluation. Each patient was given a health diary for self-assessment and completion on daily basis for an initial period of 3 months prior to the onset of drug administration. This self-assessment continued for the entire sludgy period. The health diary contains 6 symptoms and questions (e.g. headache, pains on different body parts, dizziness, fever, loss of appetite, hospitalization, school absenteeism, etc).

The primary outcome measures in physician's evaluation was related to duration, frequency and severity of attacks in FMF patients.

Patient inclusion criteria

1) Patient aged 2–15 years of both genders

2) Active patients with a diagnosis of FMF according to Tel-Hashomer criteria for the diagnosis of FMF and Tel-Hashomer key to FMF severity score, without colchicino-therapy.

• *Patient exclusion criteria:*

1) Patient suffering from infection (ARI, pneumonia, sepsis etc.) chronic illnesses such as diabetes, cardiovascular diseases, rheumatoid arthritis, kidney or liver diseases

2) The use of colchicine some time in whole lifetime

3) The use antibacterial and non-specific anti-inflammatory preparation by the patients

4) Allergy to herbal drugs

Selection of patients

Twenty-nine patients initially volunteered for this study. Twenty-four of these patients completed the pretrial assessment and met the inclusion criteria. These patients were then monitored till the end of the trial. According to the Tel-Hashomer key, moderate form was diagnosed in 19 patients and 10 patients had mild form of FMF. However 5 patients changed their mind to participate the study after first control visit.

Randomization procedure

The patients were randomized to one treatment group and the placebo group using Simple Randomization Procedure. Each jar was given a sequential number (1, 2, 3..) with the code concealed to the investigator. The sequential numbers were matched with the order of arrival of the patients.

Efficacy parameters

They were evaluated by:

– Clinical assessment
– Patient's self-assessment
– Laboratory assessment

• *Patient's self-assessment:*

The evaluation of efficacy of the treatments was based on a number of symptoms attacks (see Table 1). The patients were asked to self-evaluation the severity of symptoms, before and after treatment, as show below, after explanation and instruction by the investigator.

Table 1. CRF: Symptoms score form-patient's self-evaluation in the presence of physician after applanation and instruction by physician.

Symptom form	Assessment score
1. fever	38° – 1; 39° – 2; 40° – 3
2. abdominal pain	severe-2, mild-1, absense-0
3. chest pain	severe-2, mild-1, absense-0
4. arthropathy	chronic-3, severe-2, arthralgia-1
5. myalgia	1, absense-0
6. erysipelas-like erythema	1, absense-0

Table 2. CRF: characteristic of FMF attacks tendency score form: clinical evaluation (not presented to the patient).

Characteristic of attacks	Assessment score
Duration	
Less than 6 hours	1
6–24 hours	2
24–72 hours	3
72 hours and more	4
Frequency	
0–1 in month	1
2–3 in month	2
4 and more	3
Severity	
T = 38 °C abdominal and one-side chest pains +	1
T = 38–39 °C abdominal and two-side chest pains ++	2
T = 39–40 °C abdominal and two-side chest pains and pericarditis +++	3

• *Physicians evaluation:*

Attacks features score form: in order to test the usefulness of the physician's evaluations, based on a special standardized form for the evaluation of attacks features, a given fixed score was included as displayed in Table 2.

The number of tablets taken (morning-lunch-evening) each day and duration of diseases at the time of investigation as recorded.

• *Correlation between patient's self-evaluation and physician's evaluation.*

The correlation between the change of the total score, between features of attacks before and after treatment, regarding the physician's evaluation and the corresponding change of score of the patient's self-evaluation was assessed.

• *Laboratory assessment*

Blood was taken from each patient pre-trial, at the first during trial, during the attack and at the end of the trial. This was done, first, to confirm the diagnosis of FMF; secondly, to determine the suitability of the patients for the trial in terms, to assess the possible effects of the drug on metabolism, liver function and renal function.

The non specific parameters, such as CRP, WBC count, % of segment-nuclear leucocytes and ESR were measured to confirm the diagnosis of FMF and monitor the duration of the disease.

These laboratory analyses continued monthly for the purpose of assessing the health status of these patients as well as for the purpose of comparing the values with baseline data and statistical analysis.

Statistical methods

Each patient was only identified by number and trial ID. The data were entered in the data-base (Microsoft Excel 2000 format) patient by patient. Statistical

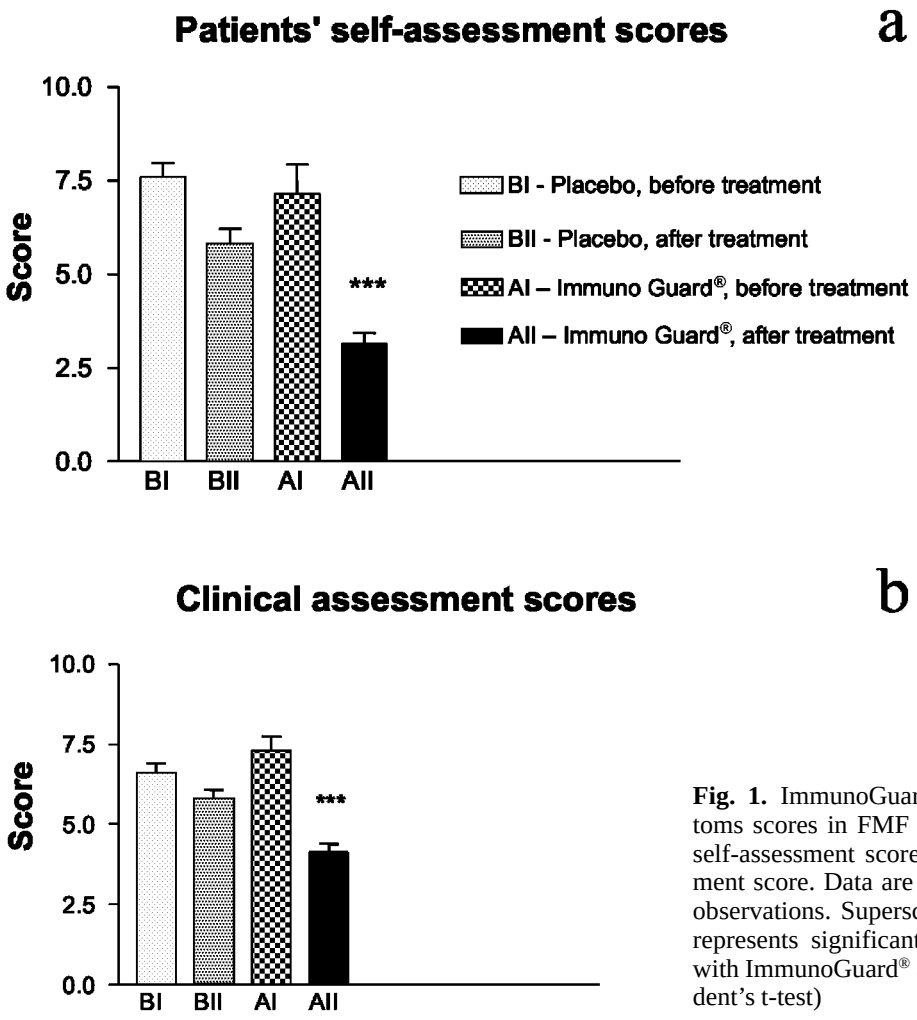

Fig. 1. ImmunoGuard causes reduction of total symptoms scores in FMF patients. Depicted in (a) is patients self-assessment score. Depicted in (b) – clinical assessment score. Data are expressed as mean ± s.e.mean of n observations. Superscripts *** indicate $p < 0.0001$, that represents significant difference between group treated with ImmunoGuard® and group treated with placebo (Student's t-test)

analysis of mean values was performed according to Student's paired and unpaired two tailed tests as well as by Mann Whitney non-parametric two tailed rank test. Data management and calculations were performed with PRISM Statistical Software Version 2.01, 1996.

Patient compliance

The compliance was ensured by the questioning the patients and collecting the jar used at the 2-nd visit. The unused tablets were counted and the lower limit for compliance was set to 93%.

Data analysis

Clinical assessment data was available from the health diary or patients for the 1–2 months of trial. Clinical data collected by clinicians during the trial were also available for analysis. The data ware fed into the computer for appropriate analysis

After an initial manual check for accuracy and completeness of the data extracted, it was transferred into computer and analysed. As an initial data exploration, the frequency distribution of all variables were produced and further examined for completeness and accuracy.

Results of monthly laboratory analyses were used to assess the health of the patients and their suitability to continue with the trial.

Type of trial

A double blind, randomized phase II trial has been fulfilled.

Results

Demographic Data

The demographic characteristics of the 24 patients, who completed the study, 10 (41,6%) were female and 14 (58,3%) were male. 14 patients were assigned to the verum group (females 7 and 7 males) and 10 to the placebo group (respectively 3 and 7).

Mean age was 7,5 years in the verum group and 8 years in the placebo group (age range 4–14 and 3–15 respectively).

Main Trial

At the end of the trial, 24 patients of the 29 pre-trial subjects had complete health diary records. These consisted of 14 patients in group A and 10 in group B. The reasons for the drop out were mainly due to the absence of attacks in the trial period.

The seven health indicators derived from the subjects' health diary reports and four indicators from the clinical assessment by clinicians were compared in the two trial groups. The summary statistics of these variables is presented in Tables 3–6 and Fig. 1, 2.

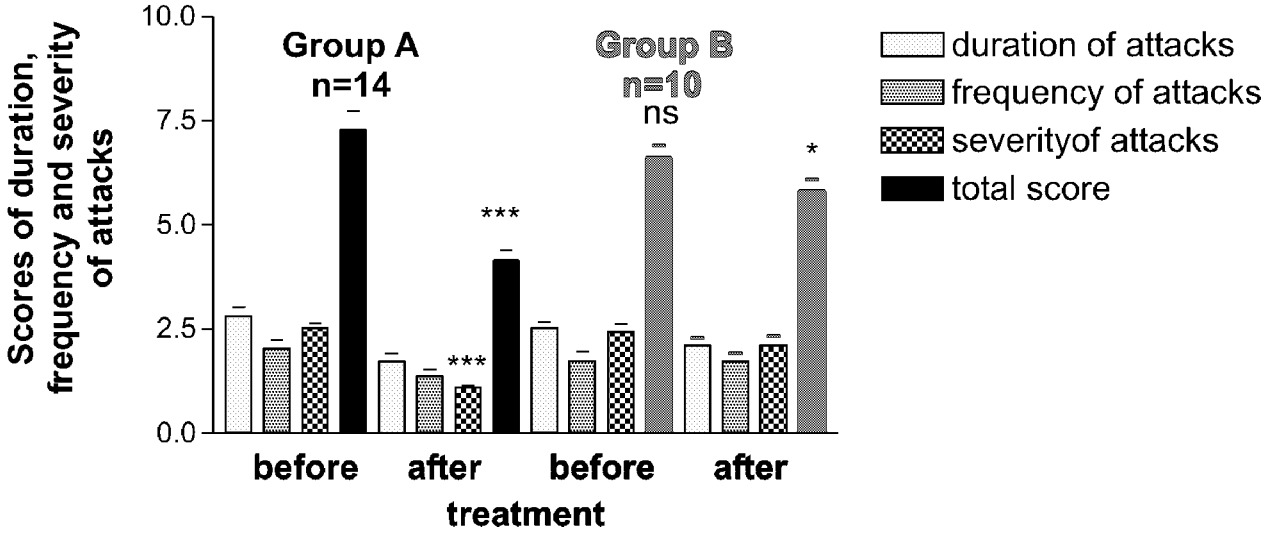

Fig. 2. Effect of ImmunoGuard® (Group A) and placebo (Group B) tablets on duration, frequency and severity of attacks in FMF patients. Data are expressed as mean ± s.e.mean of n observations. Superscripts *** indicate $p < 0.0001$, that represents significant difference between group treated with ImmunoGuard® and group treated with placebo at the end of the study (after the treatment). Superscript ns indicates $p > 0.05$, that represents no significant difference between group treated with ImmunoGuard® and group treated with placebo at the beginning of the study (before the treatment). Superscripts *** and * indicate $p < 0.0001$ and $p < 0.05$ respectively, that represents significant difference in the groups A and B respectively, before and after the treatment (Student's t-test).

Table 3. Clinical assessment results. Groups A and B.

Patients		Group A (Immuno Guard)								Group B (Placebo)							
Groups		Before treatment				After treatment				Before treatment				After treatment			
A	B	D	F	S	T	D	F	S	T	D	F	S	T	D	F	S	T
MA	SG	1.0	1.0	2.0	4.0	1.0	1.0	1.0	3.0	3.0	1.0	2.0	6.0	3.0	1.0	3.0	6.0
IG	KC	3.0	3.0	2.0	8.0	1.0	2.0	1.0	4.0	2.0	2.0	3.0	7.0	2.0	2.0	2.0	6.0
ME	CA	4.0	2.0	3.0	9.0	3.0	1.0	1.0	5.0	3.0	3.0	1.0	7.0	2.0	2.0	1.0	5.0
BL	AR	4.0	1.0	3.0	8.0	2.0	1.0	1.0	4.0	2.0	2.0	2.0	6.0	2.0	2.0	2.0	6.0
HG	AJ	3.0	2.0	2.0	7.0	1.0	1.0	1.0	3.0	2.0	1.0	3.0	6.0	1.0	2.0	3.0	6.0
AM	AA	3.0	3.0	3.0	9.0	1.0	3.0	1.0	5.0	2.0	1.0	2.0	5.0	2.0	1.0	2.0	5.0
SM	HA	3.0	3.0	3.0	9.0	2.0	2.0	2.0	6.0	3.0	2.0	3.0	8.0	2.0	3.0	2.0	7.0
TT	MA	2.0	3.0	3.0	8.0	2.0	2.0	1.0	5.0	2.0	3.0	3.0	8.0	2.0	2.0	2.0	6.0
BN	SA	2.0	2.0	2.0	6.0	2.0	1.0	1.0	4.0	3.0	1.0	2.0	6.0	2.0	1.0	1.0	4.0
VA	TK	2.0	1.0	2.0	5.0	1.0	1.0	1.0	3.0	3.0	1.0	3.0	7.0	3.0	1.0	3.0	7.0
EM		3.0	1.0	2.0	6.0	2.0	1.0	1.0	4.0								
AD		4.0	3.0	3.0	10.0	3.0	1.0	1.0	5.0								
MR		3.0	1.0	2.0	6.0	1.0	1.0	1.0	3.0								
BS		2.0	2.0	3.0	7.0	2.0	1.0	1.0	4.0								
Mean 2.786		**2.000**	**2.500**	**7.286**	**1.714**	**1.357**	**1.071**	**4.143**	**2.500**	**1.700**	**2.400**	**6.600**	**2.100**	**1.700**	**2.100**	**5.800**	
Std. Dev.		0.8926	0.8771	0.5189	1.729	0.7263	0.6333	0.2673	0.9493	0.5270	0.8233	0.6992	0.9661	0.5676	0.6749	0.7379	0.9189
Std. Er.		0.2386	0.2344	0.1387	0.4621	0.1941	0.1693	0.07143	0.2537	0.1667	0.2603	0.2211	0.3055	0.1795	0.2134	0.2333	0.2906
		Group A: Before treatment – After treatment				After treatment: Group A -Group B				Group B: Before treatment – After treatment				Before treatment: Group A-Group B			
		Paired t test				**Unpaired t test**				Paired t test				Unpaired t test			
P value		0.0003	0.0022	P < 0.0001	0.0224	0.1758	0.2164	**P < 0.0001**	**0.0003**	0.0368	1.0000	0.1934	0.0224	0.3765	0.4061	0.6908	0.2706
P value summary		***	**	***	*	ns	ns	*******	*******	*	ns	ns	*	ns	ns	ns	ns
Are means significantly different? (P < 0.05)		Yes	Yes	Yes	Yes	No	No	Yes	**Yes**	Yes	No	No	Yes	No	No	No	No

Abbreviations: D – duration; F – frequency; S – severity of attacks; T – total score; *, **, and *** indicate P < 0.05, P < 0.001and p < 0.0001 correspondingly different from controls (Student's t-test), ns– not significant.

Table 4. Patients self-assessment results. Group A

Patients' Initials	Group A													
	AI Before treatment Symptoms							AII After treatment Symptoms						
	F	AP	CP	A	M	E	T	F	AP	CP	A	M	E	T
MA	2	2	2	1	0	0	7	1	0	1	0	0	0	2
IG	2	0	2	1	0	1	6	1	0	1	0	0	0	2
ME	3	2	2	2	0	0	9	1	1	1	0	0	0	3
BL	3	2	2	0	2	1	10	1	1	1	0	1	0	4
HG	3	2	2	0	0	0	7	1	1	1	0	0	0	6
AM	3	2	2	2	2	1	12	1	1	1	0	0	0	3
SM	3	2	2	0	0	0	7	1	1	1	0	0	0	3
TT	3	2	2	0	0	0	7	1	1	1	0	0	0	3
BN	2	0	2	0	0	0	4	1	0	1	0	0	0	2
VA	2	2	2	2	0	0	0	0	1	1	1	1	0	4
EM	2	2	2	2	2	1	11	1	1	1	1	0	0	4
AD	2	2	2	0	1	0	7	1	0	1	0	0	0	2
MR	3	2	2	0	0	0	7	1	1	1	0	0	0	3
BS	2	2	2	0	0	0	6	1	1	1	0	0	0	3
Mean	**2.500**	**1.714**	**2.000**	**0.714**	**0.500**	**0.286**	**7.143**	**0.929**	**0.714**	**1.000**	**0.143**	**0.143**	**0.000**	**3.143**
Std. Deviation	0.519	0.726	0.000	0.914	0.855	0.469	2.958	0.267	0.469	0.000	0.363	0.363	0.000	1.099
Std. Error	0.139	0.194	0.000	0.244	0.228	0.125	0.790	0.071	0.125	0.000	0.097	0.097	0.000	0.293
Mann Whitney test Columns compared	Group A: Before treatment – After treatment (AI-AII)							After treatment: Group A-Group B (AII-BII)						
P, Two tailed	P < 0.0001	0.0007		0.1351	0.4301		0.0002	0.0002	0.0012		0.0132			0.0004
summary of P	***	***		Ns	ns		***	***	**		*			***
P < 0.05	**Yes**	**Yes**		**No**	**No**		**Yes**	**Yes**	**Yes**		**Yes**			Yes
Mann Whitney U	0.0000	24.00		66.00	81.00		17.50	6.500	15.00		28.00			8.500
	Paired t test							Unpaired t test						
P value						0.0003		P < 0.0001			0.0419			P < 0.0001
summary of P						***		***			*			***
P < 0.05						**Yes**		**Yes**			**Yes**			**Yes**

Abbreviations: F – Fever (38° – 1, 39–39° – 2, 39–40° – 3 scores); AP – abdominal pain; CP – chest pain; A – arthropathy; M – myalgia; E – erysipelas-like erythema
*, **, and *** indicate P < 0.05, P < 0.001and p < 0.0001 correspondingly different from controls (Student's t-test), ns – not significant.

Table 5. Patients self-assessment results. Group B.

Patients' Initials	Group B													
	BI Before treatment Symptoms							BII After treatment Symptoms						
	F	AP	CP	A	M	E	T-AI	F	AP	CP	A	M	E	T-AII
SG	2	2	2	2	1	0	9	2	2	2	2	0	0	8
KC	2	2	2	2	0	0	8	2	2	1	9	0	0	6
CA	2	2	2	0	0	0	6	2	1	1	0	0	0	4
AR	2	2	2	0	0	0	6	2	1	2	0	0	0	5
AJ	2	2	0	2	0	1	7	2	2	0	1	0	1	6
AA	1	2	2	2	0	0	7	1	1	1	1	0	0	4
HA	2	2	2	2	1	0	9	1	2	2	1	0	0	6
MA	2	2	2	2	1	0	9	2	2	1	2	0	0	7
SA	2	2	2	0	0	1	7	1	2	2	0	0	0	5
TK	2	2	2	2	0	0	8	2	2	2	1	0	0	7
Mean	**1.900**	**2.000**	**1.800**	**1.400**	**0.300**	**0.200**	**7.600**	**1.700**	**1.700**	**1.400**	**1.700**	**0.000**	**0.100**	**5.800**
Std. Deviation	0.316	0.000	0.632	0.966	0.483	0.422	1.174	0.483	0.483	0.699	2.669	0.000	0.316	1.317
Std. Error	0.100	0.000	0.200	0.305	0.153	0.133	0.371	0.153	0.153	0.221	0.844	0.000	0.100	0.4163

Mann Whitney test		
Columns compared	Group B: Before and after treatment	Befor treatment: Group A-Group B (AI-BI)
Two- tailed P value	0.0089	0.6374
Exact or approx. P value?	Exact	GA
P value summary	**	ns
Are medians significantly. different? (P < 0.05)	**Yes**	**No**
Mann-Whitney U	16.00	61.50
	Paired t test	Unpaired t test
P value	P < 0.0001	0.6492
P value summary	***	ns
Are means significantly. Different? (P < 0.05)	Yes	No

Abbreviations: F – Fever (38° – 1, 39–39° – 2, 39–40° – 3 scores); AP – abdominal pain; CP – chest pain; A – arthropathy; M – myalgia; E – erysipelas-like erythema
*, **, and *** indicate P < 0.05, P < 0.001and p < 0.0001 correspondingly different from controls (Student's t-test), ns- not significant.

Patient self-assessment

Before the trial there was no significant difference (Table 5) between groups A and B: the mean total score of self-assessment of the patients in the group A was 7.143 (Table 4, Fig. 1a) and in the group B – 7.6 (Table 5, Fig. 1a). After one month of the trial the total scores were 3.143 in the group A (significant difference before and after treatment, $p = 0.0002$, Table 4, Fig. 1a), and 5.8 in the group B (significant difference before and after treatment, $p = 0.0089$, Table 5, Fig. 1a). The total score of the severity of attacks in self-evaluation test after the treatment in the group A was significantly lower then in the group A than in group B ($p = 0.0004$, Table 4, Fig. 1a), indicating on beneficial effects of ImmunoGuard® in FMF patients. ImmunoGuard® significantly relived abdominal and chest pain, reduced the fever, arthropathy, myalgia and erysipelas-like erythema (Table 4).

Clinical assessment

Before the trial the mean scores of frequency, duration and severity of attacks in clinical assessment of the patients were not significantly different in the groups A and B (Table 3, Fig. 1b, 2). After one month of the trial significant difference between groups A and B was observed in severity of attacks (1.071 vs. 2.1, $p < 0.0001$, Table 3, Fig. 2) and in total scores (4.143 vs.5.8, $p = 0.0003$, Table 3, Fig. 1b,2). These data confirms patients' self-assessment results, indicating that ImmunoGuard® eases the severity of recurrent attacks.

Side Effects

No side effects were observed. Compliance to the test medication was over 93%

Laboratory assessment

Of the 24 patients who completed the clinical trial, 23 patients had complete laboratory results. These consist of 14 patients in group A and 9 patients in group B. Laboratory results obtained at the end of the trial, has shown, that group A significantly differs from the group B by the ESR, and CRF but did not differs by other measured parameters (WBC count, Segment-nuclear leucocytes) (Table 6). Table 6 shows that ImmunoGuard® vs placebo significantly reduced the erythrocytes sedimentation rate and C-reactive protein content in the blood of FMF patients.

Discussion

The pre-trial study shows, that the two groups were similar in term, or severity, frequency and duration of FMF, particularly as there were no significant differences in respect of the measured clinical variables in the pre-trial period.

The main study shows significant differences between patients who were on ImmunoGuard® and those who were on placebo in the most of the measured parameters.

Patients on ImmunoGuard® had significantly less episodes of FMF attacks in comparison with those on Placebo. The attacks were short, lasting for 1–3 days, and resolve without any treatment. Between the attacks the patients were active and sterling until the next episode.

The symptoms included fever, abdominal pain, chest pain, myalgia, arthropathy, erysipelas-like erythema.

The symptoms dynamics such as fever, abdominal chest pain, myalgia arthropathy, erysipelas-like erythema are showing that there is a considerable difference between the group A and B in favor of beneficial effect in the group A for all observing symptoms. ImmunoGuard® was well tolerated by the patients. The drug significantly reduced severity and duration of attacks in FMF (on the average 1,5–2 time) and insignificantly on the degree and frequency compared with product B (placebo). This conclusion especially concerns of patients with moderate disease (according to Tel-Hashomer key to FMF severity score), and cases with prevalent chest pain. In conclusion the present study have demonstrated that ImmunoGuard® definitely reduced severity of attacks in FMF in children. It reduces erythrocytes sedimentation rate and C-reactive protein content in the blood of patients ImmunoGuard® has positive influence on the course of this disease. ImmunoGuard® may therefore be presumed to be effective in preventing and treatment of active FMF.

ImmunoGuard® did not induce any significant changes in any of the measured nutritional and metabolic analyses: total protein, albumin, total calcium, fasting total cholesterol, fasting triglyceride, fasting blood glucose and uric acid. Uric acid is a measure of nucleic acid metabolism. It may be assumed that ImmunoGuard® is not cytotoxic, at least on a short-term basis.

ImmunoGuard® did not produce any significant changes in the activities of liver enzymes. AST and ALT levels are good indicators of acute and toxic damage to liver cells (Zimmerman, 1984). Therefore, it could be said that ImmunoGuard® does not produce acute toxicity to the liver. However, a longer period of study will be necessary to make a definite conclusion.

Table 6. Laboratory analysis results. Groups A and B.

Patients		Group A (Immuno Guard)											
Groups		Before treatment				Attack				After treatment			
A	B	CRP	L	S	ESR	CRP	L	S	ESR	CRP	L	S	ESR
MA	SG	–	6.0	60	27		5.6	57	30	–			
IG	KC	–	7.8	60	15	++	15.6	77	14	–	12.8	70	13
ME	CA	–	7.0	54	13					–	5.4	58	5
BL	AR	–	6.5		10	+++	12.2	70	20	–	5.4	56	10
HG	AJ	–				+	10.4	55	17	–			
AM	AA	+++	7.0	63	8	+++	7.0	63	33	–	7.0		8
SM	HA	–	5.7	56	10	++++	8.3	63	30	–	11.2	70	16
TT	MA	–				+	12.6	70	25	–	5.0	52	10
BN	SA	–	7.9	45	12	+++	8.0	63	12	–	7.5	45	8
VA	TK	–	5.4	54	7	+++	6.0	55	18	–	7.2	55	6
EM		–	10.9	52	8	+	10.0	64	18	–			
AD		–	7.4	53	14	+++	7.5	53	13	–	7.2	55	10
MR		–	5.9	50	12	+++	14.5	70	15	–	5.2	64	8
BS		–	9.2	70	16	+++	10.4	74	23	–	5.0	59	10
AA						+++	15.6	77	17	–	6.5	59	11
Mean		**0.20**	**7.22**	**56**	**12.7**	**2.538**	**10.3**	**65**	**20.4**	**0.13**	**7.12**	**58.**	**9.58**
Std. Dev.		0.77	1.59	6.8	5.35	0.967	3.41	8.2	6.80	0.52	2.49	7.4	2.97
Std. Er.		0.20	0.46	2.1	1.54	0.268	0.91	2.2	1.82	0.13	0.72	2.2	0.86

Columns compared	Group A: Before treatment – After treatment Paired t test				After treatment: Group A -Group B **Unpaired t test**			
	CRP	L	S	ESR	CRP	L	S	ESR
P value	P < 0.0001	0.4151	0.1349	0.2783	0.0045	0.1561	0.1561	0.0429
P value summary	***	ns	ns	ns	**	ns	ns	*
Are means significantly different? (P < 0.05)	Yes	No	No	No	Yes	No	No	Yes

Abbrevations: CRP – C-reactive protein; L – Leukocytes (109/l); S – percent of segmento-nuclear leukocytes; ESR – erythrocytes
*, **, and *** indicate P < 0.05, P < 0.001and p < 0.0001 correspondingly different from controls (Student's t-test), ns– not significant.

ImmunoGuard® significantly reduced the episodes of FMF attacks. This preparation did not produce any acute toxicity to the liver and the kidney. No serious side effects were observed.

The mechanism of action of ImmunoGuard® can be associated with effects of andrographolide on IFN-γ, TNF-α and neopterin production (Panossian et al. 2002). It has been shown that andrographolide increases spontaneous production of IFN-γ in whole blood cell culture. Meanwhile the efficacy of interferon alpha on colchicine-resistant familial Mediterranean fever attacks was demonstrated in several studies (Tankurt et al. 1996; Tunca et al.1997; 2000).

Andrographolide increases TNF-α production in PHA stimulated whole blood cells culture (Panossian et al. in press 2002). It has been shown that FMF patients produced significantly less TNF than controls, whether production was 'spontaneous' or induced by either LPS or PHA (Schattner et al. 1991; 1996). It can be suggested that capability of ImmunoGuard® to activate weakened ability of monocytes of FMF patients to produce TNF-α is associated with its beneficial effect on FMF patients.

In addition to *Andrographis paniculata* tree other herbal ingredients are included in ImmunoGuard: *Eleutherococcus senticosus* Maxim., *Schizandra chinensis* Bail., and *Glycyrrhiza glabra* L. special extracts standardized for the content of Eleuteroside E, Schisandrins and Glycyrrhizin. All of them are well know as antiinflammatory agents (Jung, et al. 1997;

Group B (Placebo)

Before treatment				Attack				After treatment			
CRP	L	S	ESR	CRP	L	S	ESR	CRP	L	S	ESR
–	10.0	50	12	+++	9.6	61	16	–			
+	5.3	44	15	+++	7.0	65	14	–	8.9	45	16
				++++	17.8	78	30	–	5.4	55	10
+	8.0	55	20	+++	19.7	90	20	+	5.8	56	15
+	5.8	53	10	+	5.3	51	43	+++	8.2	55	26
–	8.2			+	10.7	63	20	–	5.6	58	10
+++	7.0	56	10	++++	14.8	69	36	++	9.4	49	13
–				++++				–			
–	8.3	54	16	++++	8.3	54	22	++	16.0	77	17
–	7.0	55	8	++	7.5	55	9	–	9.8	57	11
0.67	7.45	52	13.0	2.900	11.2	65	23.3	0.80	8.64	56	14.7
1.00	1.50	4.2	4.20	1.197	5.07	12	10.9	1.13	3.47	9.4	5.28
0.33	0.53	1.6	1.59	0.379	1.69	4.2	3.65	0.36	1.23	3.3	1.87

Group B: Before treatment – After treatment

Paired t test

CRP	L	S	ESR
0.5000	0.4011	0.2079	0.4979
0.0852	ns	ns	ns
ns	No	No	No

Before treatment: Group A -Group B

Unpaired t test

CRP	L	S	ESR
0.1926	0.4589	0.1226	0.2869
ns	ns	ns	ns
No	No	No	No

separation rate (mm/hr)

Lebedev, 1971; Lupandin and Lapajev, 1981; Ohkura et al.1990; Pavlushchenko,1981; Shadrin et al. 1984; Elkin et al. 1984; Gagarinova et al. 1995; Kalashnikov, 1984; Kupin et al. 1986; Protasova and Zykov, 1984; Savenko and Tsvetkov, 1996; Fujita et al. 1980). It can be suggested that these ingredients can also contribute in anti-anti-inflammatory activity of ImmunoGuard in FMF.

The preliminary conclusion of this pilot study is that ImmunoGuard® is a safe and efficacious phytomedicine for the prophylactic management and treatment of patients with FMF. Bearing in mind the rather limited size of the study, we propose to perform a larger clinical trial of ImmunoGuard® in order to confirm this conclusion.

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