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Efficacy of a polyphenolic extract from silver fir (*Abies alba*) bark on psoriasis: a randomised, double-blind, placebo-controlled trial

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Silver fir (*Abies alba*) bark extract contains a mixture of bioactive polyphenols. We tested their effectiveness in the treatment of psoriasis in order to further investigate the potential topical anti-inflammatory activity of polyphenols by means of a randomized, double-blind, placebo-controlled add-on clinical trial, after having examined their ability to downregulate the expression of IL-1β cytokine in monocyte/macrophage primary cell culture. 61 patients with mild psoriasis met the inclusion criteria and were willing to comply with protocol requirements, were enrolled in the study. The severity of the disease was measured by psoriasis area severity index (PASI). Treatment efficacy was evaluated by assessing erythema (E, 0 to 4-point scale), desquamation (D, 0 to 4-point scale) and induration (I, 0 to 4-point scale) of lesions before and after the treatment. All patients enrolled in the study had symmetrical psoriasis plaques on the skin. All patients received O/V ointment with 2% of silver fir bark extract and/or placebo, respectively. We compared medications by right/left intra-patient comparison, so that the control group was always contralateral of the tested one. Location of the tested or control site was randomised, using a computer-generated randomisation schedule. Silver fir extract was well-tolerated. A superiority of active treatment above placebo, based on the clinical investigational PASI score system was observed by 15 % in all volunteers and in 40% regarding the improvement of psoriasis on elbows. However, statistical analysis showed no significant differences between placebo and active treatment with the extract from silver fir bark ($p < 0.05$).

1. Introduction

Psoriasis is a common chronic, immune-mediated, inflammatory systemic disease affecting predominately the skin, but also nails and joints (Griffiths and Baker 2007; Raychaudhuri and Faber 2001). It affects 2-3% of the Caucasian population (Raychaudhuri and Faber 2001; Rachakonda et al 2014) and can significantly impact quality of life. Patients with psoriasis are at increased risk of a variety of medical conditions (immune-mediated inflammatory conditions, malignancy, metabolic syndrome and obesity, myocardial infarction). Social isolation can contribute to increased risk comorbidities as well as to decreased quality of life (Weigle and McBane 2013). Diagnosis of psoriasis is primarily based on clinical features with erythematous scaling plaques of varying sizes being the most typical clinical presentation of the disease of the skin. It varies greatly in clinical presentation, usually affecting skin of scalp, knees and elbows, but typical plaques can be seen anywhere else. The eruption of psoriasis may cause numerous symmetrical plaques affecting both sides of the body or may be limited to one or two patches. Psoriasis can be divided into mild, moderate or severe, according to the psoriasis-affected body surface area (BSA), calculated Psoriasis Area Severity Index (PASI) and/or impact on quality of life, measured by Dermatological Life Quality Index (DLQI). Psoriasis is characterized by epidermal hyperproliferation, abnormal keratinocyte differentiation, inflammatory cell infiltration and angiogenesis (Machado-Pinto et al. 2016) and it tends to persist throughout life (Stern et al. 2004; Basko-Plluska and Petronic-Rosic 2012).

Abbreviations: ABI, silver fir bark extract; MPBE, maritime pine bark extract, PASI, psoriasis area severity index; NHK, normal human keratinocytes; PEG, polyethylene glycol; IL, interleukins, TNFα, tumor necrosis factor α; PK, psoriatic keratinocytes; IFN-γ, interferon gamma; ICAM-1, Intercellular Adhesion Molecule 1; NF-KB, nuclear factor kappa-light-chain-enhancer of activated B cells; BSA, body surface area

Management of psoriasis depends on the severity of the disease. In mild form topical treatment is the first line management, with emollients, keratolytics, corticosteroids, tars, calcipotriol, dithranol or tazarotene (Mason et al. 2001; Mason et al. 2013). For more generalised psoriasis, systemic therapy like methotrexate, acitretin, ciclosporine and biological medicines that target different mediators in inflammation such as adalimumab, etanercept, infliximab or ustekinumab are used (Mrowietz 2015). There is no satisfactory cure for psoriasis as both topical and systemic treatment regimens are only partially effective and can cause irritation and/or serious adverse effects like decline in renal function, hypertension, nephrotoxicity and hepatotoxicity (Mrowietz 2001; Baker et al. 2013). Patient responses vary also according to individual characteristics. In order to minimize the side effects and to test some alternative treatments, many patients are prone to use phytotherapy (Paulsen et al. 2005). According to Baron et al. (2005), approximatelly 50% of psoriasis sufferers in Europe and the USA are using herbal products, often in conjunction with official anti-psoriatic pharmacotherapy. Such herbal products can be administered orally or used topically in the form of ointments, sprays, bath, creams, gels, washes or even as a powder. Experimental studies on the antipsoriatic activity of orally administered extracts show that three plants, *Oldenlandia diffusa*, *Rehmannia glutinosa* and *Salvia miltiorrhiza* have revealed some potential anti-inflammatory and anti-proliferative activities (Deng et al. 2014). Recently, the improvement in DLQI Index was observed after oral supplementation of psoriatic patients with the french maritime pine (*Pinus maritima*) bark

extract (Belcaro et al. 2014). Among the most recommended herbal compositions are the following formulae: **Oleum horwathiensis** that is composed of *Achillea millefolium*, *Allium sativum*, *Urtica dioica*, *Calendula officinalis*, and *Taraxacum officinale*; **Capsaicin formula** contains, capsaicin from *Capsicum frutescens* that is a potent activator of cellular apoptotic factors. The formula is used as an 0.025% of topical ointment or cream; **Furocumarins formula** is composed of a mixture of furocumarins from various plants and their anti-psoriatic activity depends on the fotoactivation by means of UV-A; **Curcumin formula** contains a turmeric extract with curcumin (*Curcuma longa*), and **Tars formula** is composed of lypophillic extracts from *Betula pendula*, *Fagus sylvaticus*, *Quercus rubor* and *Juniperus communis* (Gabr and Alkhadir 2014). Besides, some hydrophillic extracts a broad specturm of polyphenols have been utilized in complementary European medicine during centuries. It has been shown that natural polyphenols possess anti-inflammatory and antiproliferative effects (Curin and Andriantsitohaina 2005). Plant polyphenols have beneficial effects in cardiovascular disease, diabetes mellitus, rheumatism, chronic venous insufficiency and other inflammatory diseases (Enseleit et al. 2012; Gulati 2014; Maimoona et al. 2011). Aditional studies were performed on normal human (NHK) and psoriatic (PK) keratinocytes with a bark extract of the conifer tree *Picea mariana* and showed that it had strong antioxidant and anti-inflammatory properties (Garcia Perez et al. 2012). These studies have demonstrated that polyphenols extracted from the bark have antioxidant properties and with no toxicity to keratinocytes. Silver fir bark extract (ABI) contains a complex mixture of bioactive polyphenols from the trunk of the silver fir tree (*Abies alba*), of which the main constituents are catechins, phenolic acids and lignans. Extracts

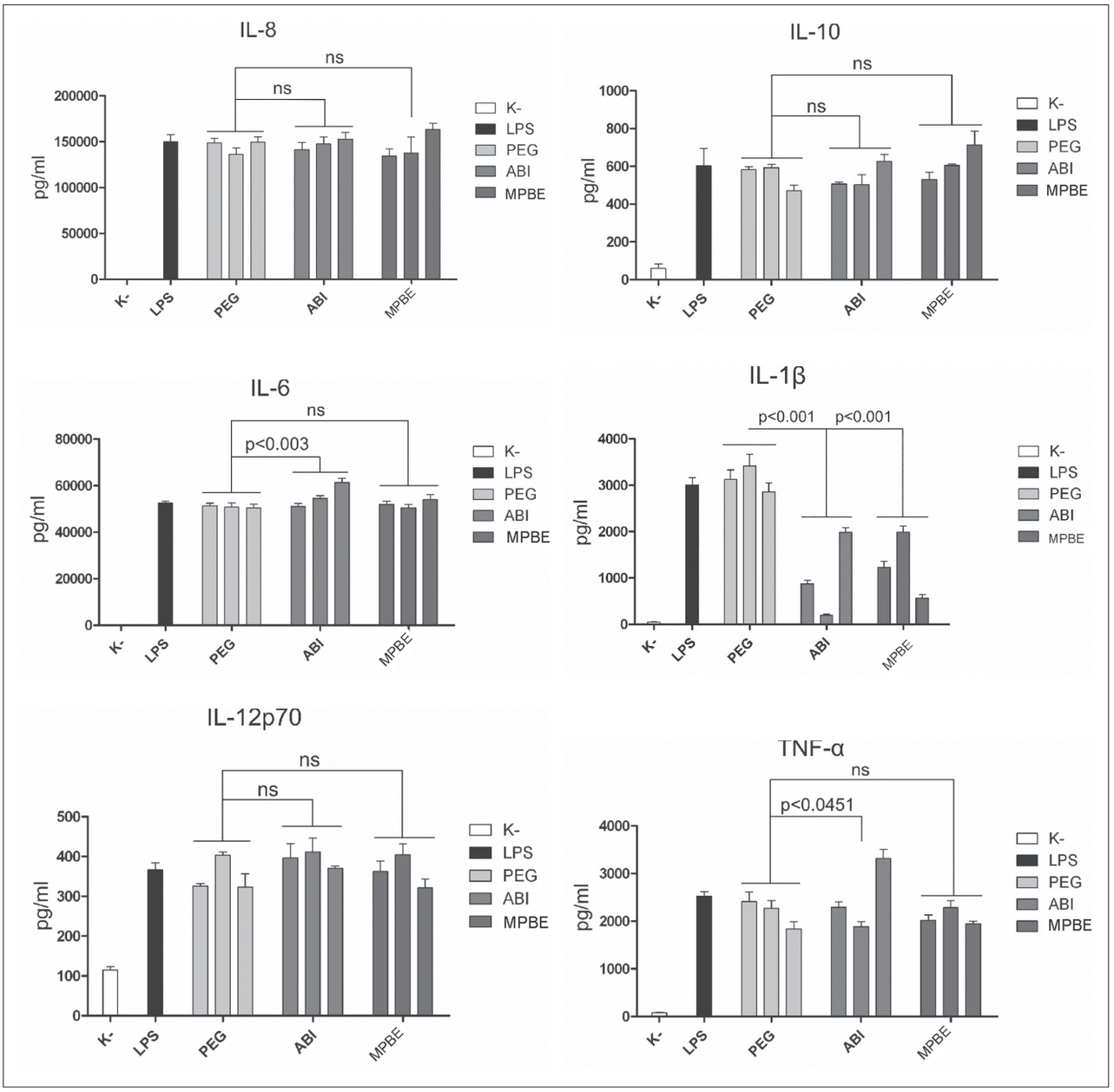

Fig 1: ABI causes significant down-regulation of IL-1β production from stimulated human monocytes/macrophages. All other measured interleukins (IL-8, IL-10, IL-6, IL-12p70, TNFα were not statistically up- or downregulated. For the experiment, cell cultures were treated with vehicle (PEG) for controls, or treated with various concentrations of ABI (1 µg, 12.5 µg, 25 µg) and Maritime pine bark extract (MPBE) as depicted in the figure. After treatment, LPS was added to the cell cultures for 48 hours. Afterwards, cell culture supernatants were taken and the concentration of interleukins was determined by BD cytokine bead array. The results are shown as mean±SD of seven independent experiments. Statistical significance was determined by ANOVA comparing two groups. A p value of less than 0.05 is considered statistically significant

from white fir exhibited strong in vitro (Vasincu et al. 2013) and ex vivo antioxidative activity (Tavčar Benković et al. 2014a), and also anti-atherogenic activity (Drevenšek et al. 2015).

In the present study, we investigated the capacity of polyphenolic extract from silver fir (Abies alba) bark (ABI) on the anti-inflammatory effects by using peripheral human monocytes/macrophages. The levels of inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF-α) were analysed in cell culture supernatants after pre-treatment of cells with the ABI and 48 hour stimulation of cells with LPS. In order to further investigate the potential topical anti-inflammatory activity of polyphenols extracted from silver fir bark, we tested their effectiveness in the treatment of psoriasis.

2. Investigations and results

2.1. Effect of ABI on the differential expression of inflammatory cytokines

The levels of inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF-α) were analysed in cell culture supernatants after pre-treatment of cells with ABI and 48 h stimulation of cells with LPS. While ABI significantly decreased the production of proinflammatory cytokine IL-1β, it had little or no effects on the production of other cytokines (Fig. 1). Similar results were obtained by maritima pine bark extract, known as pycnogenol (MPBE). Based on the findings on the cell level, we conducted the intervention clinical study.

2.2. Effect of ABI on the psoriasis clinical signs

61 patients entered the study and 56 completed it (91.8 %). The demographic and clinical characteristics of the patients at baseline are summarised in *Table 1*. Patients were interviewed and re-checked on the basis of their medical records. Only 16.4% revealed a concomitant psoriatic arthritis and all (100%) were subjected to previous treatment with topical corticosteroids, 9.8% previously received systemic therapy and 37.7% were previously subjected to phototherapy. The overall PASI score was ranging between 0.6 to 7.4.

Table 1: Patients´ demographics and baseline characteristics

Gender	Age (years)	Duration of psoriasis (year)
Male: 27 Female: 34	22-84 Mean (±SD): 53 ± 15	1 to 53 Mean (±SD): 19 ± 15
Concomitant psoriatic arthritis (proved by rheumatologist):		Yes, 10 (16.4%) No, 51 (83.6%)
Family history of psoriasis:		Yes, 28 (45.9%) No, 33 (54.1%)
Arterial hypertension therapy:		Yes, 19 (31.1%) No, 42 (68.9%)
Known hyperlipidemia:		Yes, 10 (16.4%) No, 51 (83.6%)
Smoking habit:		Yes, 17 (27.9%) No, 44 (72.1%)
Known allergies:		Yes, 13 (21.3%) No, 48 (78.7%)
Psoriasis improvement in the summer (partial or complete):		Yes, 43 (70.5%) No, 18 (29.5%)
Previous treatment history (No, %):		Topical corticosteroids 61 (100%) Systemic therapy 6 (9.8%) Phototherapy 23 (37.7%)
PASI score (at the beginning of the study):		Range: 0.6 to 7.4 Mean (±SD) 3.0 ± 1.6

The outcome measures at baseline and after 12 weeks of treatment are summarized in Table 2. Three basic parameters were evaluated: erythema, desquamation and induration. Same parameters mentioned above were measured including all patients (56 patients) and patients with psoriasis on elbows (40 patients). Within the first group we found 15% of superiority of active treatment compared with placebo and within the group of patients suffering from psoriasis on elbows, slight improvements were found by using active treatment, however the differences between placebo and active treatment were not significant ($p < 0.05$).

Table 2: Psoriasis clinical signs (erythema, induration and desquamation) scores before and after treatment on the side of the body treated with placebo and silver fir bark extract (ABI).

			Erythema	Induration	Desquamation	Sum of symptoms	Improovement during treatmetnt	Superiotity of active treatment above placebo (%)
All volunteers (n=56)	ABI	before treatment	1.64	1.32	2.14	5.11		
		after treatment	1.55	1.30	1.84	4.70	0.41	
	Placebo	before treatment	1.68	1.34	2.02	5.04		
		after treatment	1.54	1.39	1.75	4.68	0.36	15
Psoriasis on elbows (n=40)	ABI	before treatment	1.53	1.23	2.15	4.90		
		after treatment	1.48	1.18	1.73	4.38*	0.53*	
	Placebo	before treatment	1.55	1.28	2.00	4.83		
		after treatment	1.45	1.33	1.68	4.45	0.38	40

Asterisk (*) means significant ($p < 0.05$) difference to baseline. The differences between placebo and active treatment were not significant

3. Discussion

The treatments available for psoriasis have increased rapidly in recent years, predominantly due to the introduction of modern recombinant biological medicines. However, they are still incomplete and reveal some severe side effects. Although there are many drugs for different types of psoriasis, no drug can cure this disease. Several plant extracts have been used for treatment of psoriasis. The herbal sources are currently getting more reliability due to their safety and easy availability.

In the study presented, we selected a silver fir bark extract due to preliminary unpublished observations on the positive healing effect of the silver fir bark extract on scare-healing skin. Beside, from the traditional folk medicine, it was for centuries known that bark extracts from several trees had been used for treatment of psoriasis. Bito et al. (2000) demonstrated that pine bark extract down-regulates IFN-γ-induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression on HaCaT cell line. One year later, Rihn et al. (2001). performed a differential gene expression-high density filter array study in HaCaT cell lines treated by pine bark extract. It was shown that pine bark extract downregulated calgranulin A and B genes which are known to be upregulated in psoriasis. Similar results were obtained Garcia-Perez et al. (2014) with black spruce bark (*Picea mariana)* polyphenolic extract where authors demonstrated that the ethyl acetate fraction from *Picea mariana* bark extract significantly inhibited pro-inflammatory citokines through downregulation of the NF-KB pathway. Considering a modulation of the cytokine profile, we tested the polyphenolic extract isolated from the bark of silver fir (ABI) on its potential to reduce the level of proinflammatory cytokines or activate the anti-inflammatory cytokine IL-10 on isolated monocytes/macrophages. Resutls indicate that ABI mostly did not affect the expression of pro-inflammatory cytokines measured in our experiments. However, a clear effect was seen that ABI can significantly

reduce the expression of IL-1β, compared to non-treated controls. In other words, ABI had the capacity to lower IL-1β production by more than threefold. However, this effect was only seen in low to medium concentrations used in our experiments. Interestingly, when we compared two polyphenolic bark extracts, ABI and MPBE, we got very similar results that confirm the antiinflammatory activity of polyphenols, specifically targeting the IL-1 β pathway. Based on the findings presented and due to a lack of clinical data, we decided to investigate the potential anti-psoriatic effect of the silver fir extract on the clinical improvements of patients with mild to severe psoriatic symptoms. From 61 patients included into the clinical study, only 5 patients dropped out. Among them, one patient dropped out due to aggravation of psoriasis with unacceptable treatment efficacy, one patient did not attend the follow up assessment clinics, one patient dropped out because of aggravation of psoriasis and was later subjected to systemic therapy, one patient exerted eczema reaction due to application of homemade *Hypericum perforatum* herbal oil and one patient dropped out because of allergic contact dermatitis to active ingredient (silver fir bark extract) that erupted after 6 weeks of therapy.

Both extract-based and placebo-controlled formulations provided a slight improvement of the signs and the symptoms of psoriasis. The superiority of active treatment above placebo, based on the clinical investigational PASI Score system was observed by 15 % in all volunteers and in 40% regarding the improvement of psoriasis on elbows, however statistical analysis showed no significant differences between placebo and active treatment with the extract from silver fir bark. Moreover, in our study the improvement of psoriatic clinical signs on the placebo side was surprisingly low. The usual placebo effect in clinical trials with topical aplication of medicines is up to 30 % (Kreft et al. 2006). The reason could be a design of the study with left/right in patient control, or low expectations of the patients due to the natural origin of the tested substance.

This randomized, double-blind, placebo- controlled study demonstrated that silver fir bark extract in topical formulation was safe and slightly more effective than placebo in managing mild to moderate cases of psoriasis; however, statistical results did not support the use of silver fir bark extract for the management of psoriasis.

4. Experimental

4.1. Isolation of monocytes and macrophages

Monocytes and macrophages were isolated from human buffy coats (enriched leukocyte population from peripheral venous blood) obtained from the Blood Transfusion Centre of Slovenia according to institutional guidelines. Peripheral blood mononuclear cells (PBMCs) were isolated using Lympholyte®-H (Cedarlane laboratories, Ontario, Canada). The cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS), counted, and used as a source for magnetic isolation of CD14-positive cells (monocytes) (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The magnetic isolation procedure usually results in cell populations of greater than 95% purity, enabling studies aimed at specific cell types without interference from other cell populations. All cell cultures were supplemented with RPMI 1640 medium (10% fetal bovine serum (FBS), Gentamicin (50 g/ml; Gibco, Paisley, UK)). To generate macrophages, monocytes were cultured in culture medium for 5 days in the presence of monocyte-colony stimulating factor (M-CSF) (Peprotech EC, UK). During culture, half of medium was exchanged on day 2, with addition of starting quantities of M-CSF. On day 5, the resulting macrophages were washed twice in DPBS, collected, counted and used as a source for anti-inflammatory studies.

4.2. Cultivation of monocytes/macrophages with ABI and measurement of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, , TNF-α) and anti-inflammatory cytokine IL-10

Monocytes/macrophages was obtained as described. The cells were treated with various concentrations of ABI (1 µg/ml – 25 µgml in PEG) for 2-6 h. Afterwards, the cells were stimulated with LPS for two days. After two days, supernatants of cell cultures were analyzed for the presence of IL-1β, IL-6, IL-8, TNF-α, and IL-10. The BD Human Cytometric Bead Array (BD Biosciences) was used to assay the cytokine levels in the cell culture supernatant, according to the manufacturer's protocol.

4.3. Topical ointment with the standardized extract from bark of Abies alba (ABI)

Silver fir (*Abies alba*) bark extract was isolated according to the protocol developed by Tavčar Benković et al. (2014b). Topical ointment was composed by mixing the following ingredients: Lanette N 5%, stearinic acid 3,5%, cetanol 0,5%, Tegosoft CT, 3%, olive oil 5%, Cetiol SN 2%, CMC cellulose 1%, parrafinum liqidum 1%, demineralized water 72,3%, glycerol 3%, urea 1%, phenonip 0,5%, silver fir bark extract 2%, trietanolamin 0,2%. In placebo, the active ingredient-silver fir bark extract was omitted.

4.4. Trial design and setting

This randomized, double-blind, placebo-controlled study was conducted at the University Medical Centre Ljubljana, Department of Dermatovenerology. The study complied with the World Medical Association Declaration of Helsinki, Good Clinical Practice and was approved by the National Medical Ethics Committee. All patients were thoroughly informed about the study and they signed written informed consent prior to their enrolment in the study.

Adult patients with mild psoriasis (according to PASI) were enrolled in our study. Each patient was examined at the beginning of the treatment, after 4 and 12 weeks of therapy by a single clinical investigator, a clinical dermatologist. The severity of the disease was measured by the psoriasis area severity index (PASI). At the beginning of the study all enrolled patients had a PASI score under 10. Treatment efficacy was evaluated by assessing erythema (E, 0 to 4-point scale), desquamation (D, 0 to 4-point scale) and induration (I, 0 to 4-point scale) of lesions before and after the treatment, with 0 as the absence and 4 as the maximum degree of E, I or D. The mean changes in score of E, D and I were the primary outcome measures of the trial. PASI was also calculated at the beginning and at the end of the study and change in values were evaluated.

First 61 patients with mild psoriasis, who visited our outpatient clinic, met the inclusion criteria and were willing to comply with protocol requirements, were enrolled in the study. One of the inclusion criteria was location of psoriasis lesions on symmetrical sites of the body or/and extremities. The exclusion criteria were current diagnosis of moderate or severe psoriasis, pustular, generalised, guttate or erythrodermic psoriasis, the presence of any other skin condition that could have confused psoriasis assessment (e.g. atopic dermatitis, seborrhoeic dermatitis, contact dermatitis, cutaneous mycosis, etc.), pregnancy and breast-feeding, patients with known serious hepatic illness or impairment of hepatic function and the use of medications that are known to exacerbate psoriasis. All patients had 2-months wash-out period for systemic anti-psoriatic therapy or phototherapy and at least 2-weeks wash-out period for topical treatment before starting the study.

All patients enrolled in the study had symmetrical psoriasis plaques on the skin. We compared medications by using a right/left intra-patient comparison, so that the control group, allocated left or right on the body, was always contralateral of the tested one. Location of the tested or control site was randomised, using a computer-generated randomisation schedule, managed by the staff, which was not part of the clinical study.

At the beginning all patients were examined by clinical dermatologist. 61 subjects met the inclusion criteria of PASI less than 10 and expressed interest in participating in the study. In all patients one side of the body was the test side and the contralateral side was a control side, an ideal negative/positive test control due to the same phatophysiological basic level. After the wash-out period, clinical assessment and signed consensus patients entered the study and received the medication. Medications were packed in identical containers with the code and information to the patient for which part of the body is it for (left or right), kept by a staff member who was not part of clinical research. This enabled the clinical dermatologist, performing assessments, to be blinded through the whole study.

Patients were instructed to apply two different topical creams marked with side of the body (L for left and R for right), on plaques on the left or right side of the body twice daily, in the morning and in the late evening. They applied medications to all psoriatic plaques on the side of the body, investigator told them so (i.e. right and left elbow, right and left knee, right and left part of the body). Application was done without occlusion for 12 weeks or until cleared. They applied the cream by massaging it gently onto the affected areas. They were instructed not to do this just before having a bath, shower, washing that part of the body or going swimming. They were asked not to use any other psoriasis treatments concomitantly with the trial medications except plain emollients, if necessary. They were also asked not to expose the body to the sun, that's why the study was not going on in the summer season. The study started on late October and ended by the middle of May. After 12 weeks of therapy 56 patients completed the study, all were included in the final data analysis.

4.5. Statistical analysis

Descriptive statistics was used to analyse demographic data. Paired Students t test (Workpackage SSPS 19) was use to compare psoriasis symptoms between the placebo and tested group and between baseline and end of treatment.

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