TREATMENT OF ATOPIC DERMATITIS IN CHILDREN WITH BIOAPIFIT® ANTI-INFLAMATORY HERBAL OINTMENT - A PRELIMINARY STUDY

Višnja Oreščanin

OREŠČANIN Ltd., Laboratory for herbal drugs development, A. Jakšića 30, 10000 Zagreb, Croatia,

Correspondence to: prof. dr. sc. Višnja Oreščanin, senior scientist; OREŠČANIN Ltd, Laboratory for herbal drugs development, Ante Jaksica 30, 10000 Zagreb, Croatia, Tel. +385914377905; vorescan@gmail.com

Abstract

Objectives: The purpose of this work was development, formulation and testing of new herbal ointment for the treatment of mild to severe atopic dermatitis in infants and children (4 to 56 months old) as possible alternative therapy to standard corticosteroids and immunomodulants.

Patients and methods: 50 patients were treated 8 weeks (every four hours) with the ointment containing the following ingredients: *Avena sativa*, *Nigella sativa*, *Argania spinosa*, *Prunus amygdalus*, *Daucus carota*, *Helichrysum italicum*, *Calendula officinalis*, *Matricaria chamomilla*, *Bellis perennis*, *Lavandula officinalis*, *Achillea millefolium*,*Thymus serpyllum*, *Salvia officinalis*, *Symphytum officinale*, *Plantago major*, *Olea europaea*, *Melaleuca alternifolia*, *Cera alba*, honey, and glycerol. The severity of the disease prior and after the therapy was assessed by SCORAD index.

Results: The total score before the therapy ranged from 23.1 to 99.4 (66.50±23.43). Among the tested patients 76%, 20% and 4% had severe, moderate and mild symptoms, respectively. Following the eight weeks treatment the extent, intensity and subjective symptoms decreased significantly. The total score decreased more than 20 times compared to the initial values and ranged from 0 to 14 (13.18±4.29). The intensity parameters (swelling, oozing/crusting and scratch marks) as well as subjective parameters (itch and sleeplessness) disappeared completely following two months therapy.

Conclusion: Two months of the topical treatment with Bioapifit® herbal anti-inflammatory ointment resulted in complete remission of the symptoms in 56% of the patients while in another 44% of them only mild symptoms persisted. Such excellent results could be atributed to the ointment's formulation containing emollients, strong anti-inflammatory, immunomodulating, wound healing and antimicrobial agents of herbal origin that simultaneously targeted the multiple mechanisms involved in AD pathogenesis. This ointment could be used as alternative therapy to the topical corticosteroids and immunomodulants.

Key words- *atopic dermatitis, children, Bioapifit® herbal ointment, SCORAD index*

1. INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease which starts early in life (Bieber, 2010). The main symptom of AD is constant itching throughout the day which usually worsens during the night, causing insomnia, irritability, exhaustion that impairs the quality of life both the patient and his family significantly. It is often associated with other atopic diseases such as food allergies, asthma and allergic rhinitis. The symptoms of AD may vary from mild to moderate to severe forms of the disease depending of the age of the patient. It usually starts in early infancy between 2nd and 6th month of life. It was found that in 45% of children disease begins during the first six months of life, in 60% of them during the first year, and in 85% the symptoms appeared before five years of age

(Bieber, 2010). AD has a tendency of disappearance with age. In 50% of the children the symptoms disappeared before seven years of age, and in 70% before adolescence. If AD lasts until adolescence, it is very likely that the patient will have difficulties in adulthood (Bieber, 2010). The clinical feature may differ with age and severity of the disease. The acute phase is characterized by oozing, crusted, eroded vesicles or papules on erythematous plaques while in sub-acute phase thick and excoriated plaques are usually formed and the chronic phase is characterized by lichenified, slightly pigmented, excoriated plaques (Bieber, 2010).

The prevalence of AD in the last 30 years has increased up to three times (Pustišek et al., 2016). It is estimated that in the industrialized countries 15-30% of children and 2-10% of adults are affected by AD (Bieber, 2008; Bieber, 2010). The highest incidence was obtained in northern Europe (ISAAC). It was confirmed that the current prevalence of AD in developed countries is up to 20% (Deckers et al., 2012). The prevalence of AD also increased in developing countries, especially in Africa and East Asia (Flohr and Mann, 2014).

AD could be associated with elevated levels of immunoglobulin E (IgE). It was confirmed (Spergel, 2010; Carlsten et al., 2012; Dharmage et al., 2014) that AD is the first disease to appear in a series of allergic diseases such as food allergy, asthma, and allergic rhinitis, leading to the “atopic march”. Early or severe AD and cutaneous sensitization to environmental allergens may lead to subsequent allergic disease at other epithelial barriers.

Almost 30% of the children suffering from AD will develop asthma later in life, and almost 70% of them will develop allergic sensitization and the symptoms of allergic rhinitis (Dharmage et al., 2014; Gustafsson et al., 2000). High correlation between food allergy and AD was also reported (Allen and Dharmage, 2010).

Genetic and environmental factors represent the main risk factors for the development of AD (Carson, 2013). 37.9% and 50% of the children under age of four will develop AD if one and both parents have atopic disease compared to 27.1% of the atopic children with no family history of atopy (Bohme et al., 2003). Although, heredity factors are clearly linked with increased risk of AD development, the influence of environmental factors onto AD gave contradictory results (Carson, 2013).

The development of inflammation that leads to AD was connected either by primary immune dysfunction resulting in IgE sensitization or by epithelial-barrier disturbance. However, the disturbance of the epithelial barrier will lead to the immunologic dysregulation leading to the development of inflammation.

The imbalance in the T-cell subsets, especially Th2 cells will lead to the production of Th2 cell–associated pro-inflammatory, pro-allergic cytokines, primarily IL-4, IL-5, and IL-13, causing higher expression of the adhesion molecules on endothelial cells, inhibition of apoptosis of eosinophils and increased level of IgE and consequently, decreased interferon-gamma levels (Bieber, 2008, Bieber, 2010; Peng and Novak, 2015). During the chronic phase of AD, the Th0 and Th1 cells predominate resulting with elevated levels of the IFNG-γ, IL-12, IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF) as well as IL-18, IL-11, IL-17 TGF-ß1 (Bieber, 2008, Bieber, 2010). Besides, Th17 cells could be also elevated in patients with AD. However, more research was needed to understand the precise contributions of Th1 and Th17 cell to the pathogenesis of AD (Peng and Novak, 2015; Koga et al., 2008).

Moreover, eosinophils and mast cells have also significant role in the pathogenesis of AD (Molfino et al., 2011; Hershko et al., 2011). In the last few years, basophils and group 2 innate lymphoid cells (ILC2s) are connected to the pathogenesis of AD (Kim et al., 2013; Kim et al., 2014; Roediger et al., 2013; Imai et al., 2013; Salimi et al., 2013) since they are important sources of IL-4, IL-5, and IL-13 pro-allergic cytokines (Kim et al., 2013; Kim et al., 2014). Those cells were regulated by a family of epithelial cell–derived cytokines directly released from the damaged keratinocytes, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 (Kim, 2015).

The epidermal barrier dysfunction is connected to AD development due to the entrance of antigens through the damaged skin which will lead to the production of inflammatory cytokines. It was found that mutations in the gene encoding filaggrin, a key protein that facilitates terminal differentiation of the epidermis and formation of the skin barrier is the strongest known genetic risk factors for the development of AD (Osawa et al., 2011; Smith et al., 2006; Palmer et al., 2006).

Filaggrin dysfunction could lead to the release of the epithelial cell–derived cytokines, including TSLP, IL-25, and IL-33 and consequently, development of inflammation that will lead to AD

(Hvid et al., 2011; Savinko et al., 2012; Soumelis et al., 2002; Brandt et al., 2011). Aside from *FLG* mutations it was found that genetic variants of TSLP interact with mutations in filaggrin to influence AD disease persistence in patients. Among the patients with *FLG*- loss of function mutation, those with *TSLP* variation were more likely to have less persistent disease (Margolis et al., 2014).

Taking all together it was obvious that loss of filaggrin function leads to increased trans-epidermal penetration of environmental allergens, increasing inflammation and sensitivity and potentially leading to the atopic march (Kubo et al., 2012).

Although, there is no cure for AD warious treatment approaches were developed so far in order to alleviate the symptoms. The data were summarized in the work of Leung and Hon, 2015. In short, aside from continues usage of emollients and wet bandages in order to hydrate the skin and form the protective barrier, the therapy include either topical or systemic anti-allergic or immunomodulating drugs. Topical treatment include topical corticosteroids of different potency depending on the severity of the symptoms, topical immunomodulants (topical calcineurin inhibitors), topical sodium cromoglicate (anti-allergic, anti-itch, and anti-inflammatory drug). In the case of *Staphylococcus aureus* infection oral antibiotics were also included while antihistamines were sometimes prescribed in order to reliefs the itching regardless of the lack of evidence of their efficiency as anti-pruritics. In the case of chronic severe AD in children and adults that not responding to the topical therapy systemic immunomodulating agents are used. Among them, cyclosporin A, azathioprine and methotrexate are most commonly prescribed. Other approaches include phototherapy which is mostly used for the treatment of chronic lichenified forms of moderate-to-severe AD. Dietary interventions, allergen-specific immunotherapy, Chinese herbal medicine, psychological and educational interventions could be also of some help. However, more research is needed to confirm their efficiency.

Since there was no reliable biomarker for the diagnosis of AD, it is predominantly based on a constellation of clinical feature (Leung and Hon, 2015). Various tools were developed so far to assessing AD. However, the Severity Scoring of Atopic Dermatitis Index (SCORAD) is best validated and most commonly used instrument to assess the clinical signs of AD (Schmitt et al., 2013). The SCORAD index (Oranje, 2011; Oranje et al., 2007) assessing the extent (Score A), the intensity (Score B) and subjective symptoms (Score C). The total score is calculated according to the formula A/5 + 7B/2 + C with maximum value of 103. For the extant the rule of nines is applied on a front/back drawing of the patient's inflammatory lesions (Head and neck 9%, Upper limb 9% each, Lower limb 18% each, Anterior trunk 18%, Back 18% and genitals 1%). The total score A may range from 0 to 100. Six parameters (signs) were included for the assessment of the intensity (Redness, Swelling, Oozing / crusting, Scratch marks, Skin thickening (lichenification) and Dryness). Each of these signs is assessed as none (0), mild (1), moderate (2) or severe (3). B score was calculating by summing the individual results for each sign. The maximum value for score B is 18. Subjective symptoms i.e., itch and sleeplessness, are each scored by the patient or relative using a visual analogue scale (VAS) where 0 is no itch (or no sleeplessness) and 10 is the worst imaginable itch (or sleeplessness). These scores are summed to obtain Score C (maximum 20).

The purpose of this work was development, formulation and testing of new herbal ointment for the treatment of mild to severe atopic dermatitis in infants and children as possible alternative therapy to standard corticosteroids and immunomodulants.

2. PATIENTS AND METHODS

2.1. Study Design

50 patients (31 females and 19 males) in the age range from 4 to 56 months diagnosed from mild to severe atopic dermatitis whose parents rejected corticosteroid therapy were included. All the parents signed informed consent and completed the questioner including general questions about child and the parents, the onset of the disease, family history of atopy. The diagnosis and the severity of the disease were based on the SCORAD index. The patients were treated 8 weeks with the herbal ointment. The ointment was applied on affected area every four hours during the whole course of the study. It was also applied on whole body after each bathing or at least twice a day in order to moisturize the skin. The parents were advised to make daily notes about the changes of the symptoms. The parents were also advised to use soft cotton clothing next to the child’s skin, maintain

mild temperatures particularly at night, use humidifier, use mild detergent, with no bleach or fabric softener for clothes washing, provide non-smoking environment, avoid contact with animals, avoid hipper-allergenic food in the case of concomitant food allergy. Two follow-ups (after four and eight weeks) were carried out in order to assess the efficacy of the therapy.

2.2 Preparation of the Ointment

The process of the production of macerate and ointment was described in details in our previous papers (Orescanin et al., 2015 a,b; Orescanin and Findri, 2016). The final product consists of: 15% of colloidal oat (*Avena sativa*), 5% of black cumin oil (*Nigella sativa*), 5% of argan oil (*Argania spinosa*), 5% of almond oil (*Prunus amygdalus*), 2% of carrot oil (*Daucus carota*), 5% of immortelle flowers (*Helichrysum italicum)*, 10% of marigold flowers (*Calendula officinalis*), 5% of chamomile flowers (*Matricaria chamomilla*), 2% of daisy flowers (*Bellis perennis*), 2% of lavender flowers (*Lavandula officinalis*), 5% of aerial part of yarrow (*Achillea millefolium*), 2% of aerial part of wild thyme (*Thymus serpyllum*), 2% of aerial part of sage (*Salvia officinalis*), 5% of comfrey leaves (*Symphytum officinale*), 7% of plantain leaves (*Plantago major*), 4% of olive leaves (*Olea europaea*), 0.2% of tea tree (*Melaleuca alternifolia*) essential oil, 8% of bee wax (*Cera alba)*, 5% of honey, 4.8% of glycerol.

2.3. Statistical Analysis

For statistical evaluation Statistica 11.0 software package was employed. The description of the treated population was done by basic statistics and frequency tables. Statistical significance was set to $p<0.05$ in all the tests performed. The differences in the mean values of each parameter prior and after the therapy were assessed by Kruskal-Wallis ANOVA and Multiple Comparisons test. The influence of the predictor variables on the values of SCORAD index was tested by Multiple regression method and General regression model (Oreĉĉanin et al., 2015a).

3. RESULTS AND DISCUSSION

3.1 Description of the Population

The study included the infants and children in the age range from 4 to 56 months (18.48±16.54 months). The onset of the disease was from 2 to 6 months (4.24±1.93) which was in accordance with previously published data (Bieber, 2010; Pustisek, 2016). Most of the children (82%) came from the middle to upper class, mostly from high educated (76%) parents. Similar results were obtained by Pustisek, 2016. When we looked at the extent of AD (Figure 1) the head and neck was the most affected area of the body (92%), followed by upper limbs (56%), lower limbs (36%), genitals (32%), anterior trunk (24%) and back (8%). The mean values and standard deviations for the extent (Score A), intensity (Score B), subjective symptoms (Score C) and total score were presented in Fig. 2. Score A ranged from 9 to 82 (37.40±16.95), score B from 5 to 18 (12.92±.4.40), score C from 2 to 20 (13.80±5.55) and total score from 23.1 to 99.4 (66.50±23.43)

The following mean values and standard deviations were obtained for the intensity parameters (Fig. 3): 2.60±0.56 for redness, 2.24±0.78 for swelling, 1.64±0.99 for oozing/crusting, 2.28±0.84 for scratch marks, 2.04±0.79 for lichenification and 2.12±0.78 in the case of dryness. In the case of subjective factors the mean value and standard deviation for itch was 6.88±2.74 and sleeplessness 6.92±2.78.

According to the total score 76% of the patients developed severe symptoms, 20% of them had moderate symptoms while only 4% them had mild symptoms (Fig. 4).

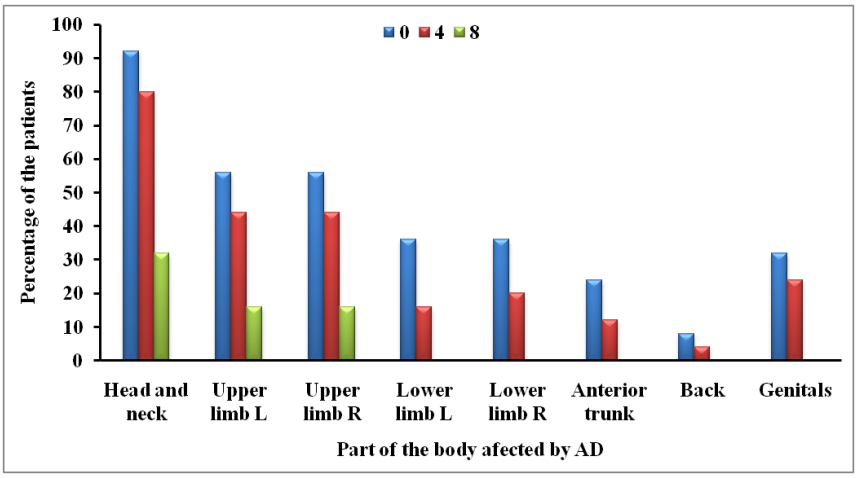

Figure 1. Percentage of the patients affected by atopic dermatitis for each part of the body assessed by SCORAD index before and following four and eight weeks of the therapy with Bioapifit® anti-inflammatory ointment

Family history of atopic diseases was obtained in 48% of the patients. Bohme et al., 2003 found significantly higher percentage of children with AD if one or both parents have atopic disease compared to those without family history of atopy. Pustisek et al., 2016 reported that among 134 participants, 41% of them had family history of atopy. In the present study 40% of the patients had also some other allergic disease. Among them food allergies prevailed (24%), followed by allergic rhinitis (8%) and asthma (8%). Atopic dermatitis was the first allergic disease that occurred in the examined children. Similar results were obtained by Pustisek, 2016 where 44% of the patients in association with AD had some other allergic disease and the highest percentage was associated with food allergies (33.6%) followed by more than two allergic diseases. Two of our participants had also diagnosis of ulcerative colitis and one of them Crohn disease. All those results support the “atopic march” theory where early or severe AD and cutaneous sensitization to environmental allergens may lead to subsequent allergic disease at other epithelial barrier surfaces (Spergel, 2010; Carlsten et al., 2012; Dharmage et al., 2014).

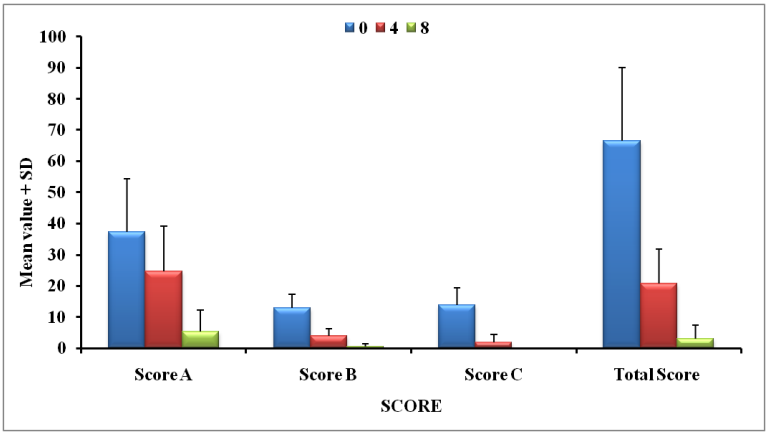

Figure 2. Mean values and standard deviations of the results of the severity scoring of atopic dermatitis (SCORAD) index assessing the percentage of the affected area of the body (Score A), intensity of the

symptoms (Score B), subjective symptoms (Score C) and total score before (0) and after 4 and 8 weeks of the treatment with Bioapifit® anti-inflammatory ointment.

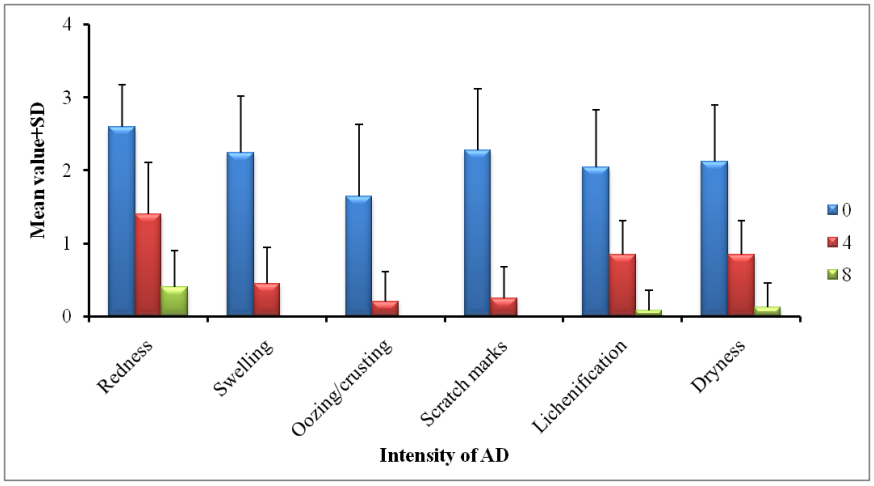

Figure 3. Intensity of atopic dermatitis symptoms assessed by SCORAD index before and following four and eight weeks of the treatment with Bioapifit® anti-inflammatory ointment.

Based on the results of multiple regression analysis there was a strong, statistically significant correlation ($R = 0.89$; $p < 0.00000$) between the total score and predictor variables (age, family history, other allergic diseases). The severity of the disease was negatively correlated with age ($\beta = -0.44$; $p<0.000630$). Indeed, the highest SCORAD index was obtained in the youngest patients < 6 months old. Statistically significant, positive correlation was obtained between SCORAD index and family history of atopic disease ($\beta = 0.57$; $p<0.000597$) as well as SCORAD index and the presence of other allergic diseases ($\beta = 0.32$; $p<0.037002$). According to the beta coefficients and their significance level the influence of the predictor variables on the result of SCORAD index increased in the following order: other allergic diseases<age<family history.

The results of general regression model expressed as Pareto charts of t-values were completely in agreement with the results of multiple regression analysis (Figure 5). All three predictor variables showed statistically significant influence in the results of SCORAD index with family history as most important predictor variable. As we mentioned previously, in our study 48% of the patients have family history of atopic diseases. Among the family members, atopic disease was most commonly found between siblings followed their mothers.

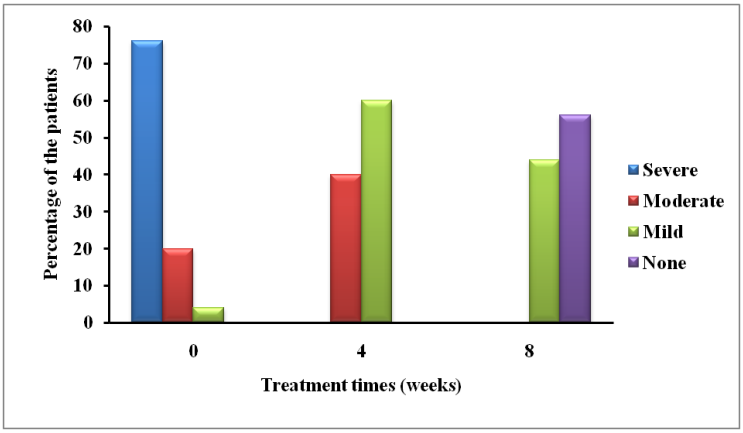

Figure 4. Percentage of the patients affected by atopic dermatitis depending on the degree of severity assessed by SCORAD index before and following four and eight weeks of the treatment with Bioapifit® anti-inflammatory ointment.

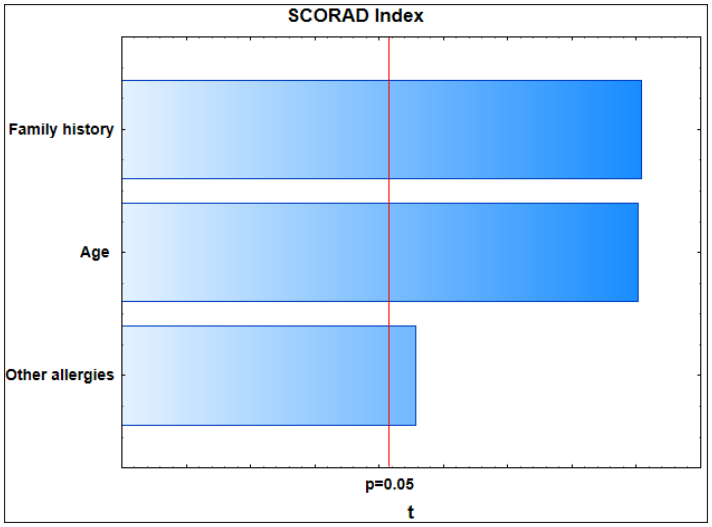

Figure 5. Pareto charts of t-values testing for the influence of predictor variables (family history, age, other allergic diseases) on the results of SCORAD index

3.2 The Response to the Therapy

Following the 30 days of the treatment with Bioapifit® anti-inflammatory ointment the mean value of the total score of SCORAD index was decreased more than three times compared to the results prior to the therapy (20.68±11.26). The mean values and standard deviations for the extent (24.72±14.58), intensity (3.96±2.32) and subjective symptoms (0.96±1.34) also decreased significantly (Fig. 2). Among the intensity parameters, the highest decrease compared to the results prior to the therapy (Fig. 3) was obtained for scratch marks (9.5 times), oozing/crusting (8.2 times) and swelling (5.1 times) while other three parameters decreased from 1.9 to 2.5 times.

According to the total score following the 30 days therapy, 40% of the patients had moderate and 60% of them had mild symptoms of AD. Itching as most important symptoms of AD disappeared completely in 60% of the patients while 40% of them graded the severity of the symptom from 1 to 3 of 10 on the VAS scale (0.92±1.29).

Prolonged treatment for another 30 days resulted in further decrease of all considered parameters of SCORAD index (Figs. 1-3) and the degree of the severity (Fig. 4). Mean value and standard deviation of the total score was 3.18±4.29 (range from 0 to 14.1) which was decreased more than 20 times compared to the period before the therapy. Only mild symptoms of AD persisted in 44% of the patients (Fig. 4).

The intensity parameters swelling, oozing/crusting and scratch marks disappeared completely while redness, lichenification and dryness decreased for 6.5, 25.5 and 17.7 times compared to the period prior to the treatment. The subjective parameters itch and sleeplessness disappeared completely following two months of the therapy.

According to the results of Kruskal-Wallis ANOVA and Multiple Comparisons test the total score as well as all SCORAD index parameters showed statisticaly significant diference amnong the initial values (before the treatment) and the values obtained after 30 and 60 days of the therapy.

Two months of the topical treatment with Bioapifit® herbal ointment resulted in complete remission of the symptoms in 56% of the patients while in another 44% of them only mild symptoms are recorded. Itching, as the basic symptom of AD disappeared in all 50 patients. Such excellent results could be ascribed to the ointment formulation containing emollients, strong anti-inflammatory, immunomodulating, wound healing and antimicrobial agents of herbal origin.

As mentioned earlier the disturbance of epidermal barrier is highly associated with the pathogenesis of AD since it allows the entrance of the allergens through the damaged skin resulting in the production of inflammatory cytokines and progression of AD (Osawa et al., 2011; Smith et al., 2006; Palmer et al., 2006). Besides, trans-epidermal water loss is also increased in AD due to skin barrier dysfunction (Kelleheretal et al., 2015). So, skin hydration and restoration of the normal function of epidermal barrier is essential in the management of AD.

It was reported that argan oil has positive effect on the skin hydration (Boucetta et al., 2014) which was demonstrated by significantly higher water content of the epidermis and significant decrease of trans-epidermal water loss following both oral and topical application. The authors concluded that argan oil improved the skin hydration by restoring epidermal barrier function and by maintaining the water-holding capacity. These was connected with the high concentration of linoleic acid in argan oil (31-35%) since this acid is the predominant constituent of the epidermal cement ceramides, more precisely, ceramide-1 subclass which plays a key role in the organization of the *stratum corneum* lipids. This beneficial effect of linoleic acid, on the improvement of skin hydration is achieved by the activation of peroxisome proliferator-activated hormone which promotes the activation of epidermal keratinocyte differentiation leading to the synthesis of the cement epidermal lipids and finally to the restoration of the normal barrier function. Argan oil based nanostructured lipid carrier hydrogel preparation exhibited promising skin-moisturizing effect (Tichota et al., 2014). Positive effect of argan oil on skin elasticity was also proven following the 60 days of the topical application (Boucetta et al., 2015) which was connected with the presence of high value of vitamin E (a lipid-soluble nutrient) which in addition to its high antioxidant potential can act on skin elasticity by down-regulation of TGF-β resulting in a signal inducing fibroblasts to produce more collagen and elastic fibers necessary for the normal appearance and integrity of the skin. Indeed, significant influence of vitamin E in the management of AD symptoms was reported by Tsoureli-Nikita et al., 2002. They found a significant decrease of IgE serum level in AD patients following the 8 months of vitamin E treatment. Furthermore, a remarkable improvement in facial erythema, lichenification, and skin general appearance was reported. The researchers concluded that eczematous lesions healed mostly as a result of decreased pruritus. Following the sixty days of the treatment of AD patients with the combination of E and D vitamins the mean value of SCORAD index decreased for 64.3% (Javanbakht et al., 2011). Beneficial effects of the combination of vitamins A, C, and E as well as E and C on the treatment of skin related diseases and conditions were also reported (Keller and Fenske, 1998; Hayakawa et al., 1981).

From the above mentioned findings it could be concluded that base oils rich in vitamins A, C, D and especially E, and polyunsaturated fatty acids, especially linoleic acid have very beneficial effect on the restoration of the normal epidermal barrier function. Keeping that in mind, 5% of almond oil was also included in the formulation since this oil has long tradition of use in skin care products (Centerchem, 2006) due to its excellent emollient, moisturizing and skin softening effect that was connected with high linoleic acid amount (20-30%) and vitamin E (282 mg/kg). Besides, between 62.0 and 86.0% of oleic acid is found in almond oil which promotes the transportation of polyunsaturated fatty acids in the skin by softening of the horny layer that promote the production of beneficial eicosanoids that play an integral part in homeostatic mechanisms related to skin health and structural integrity (Nicolaou, 2013).

2% of carrot root macerate rich in carotenoids, vitamins B, C, D, E also provide similar benefits to the impaired skin barrier.

It was found that primary immune dysfunction resulting in IgE sensitization plays the key role in the pathogenesis of AD (Bieber, 2008, Bieber, 2010; Peng and Novak, 2015). Consequently, *Nigella sativa*, the plant with well documented immunomodulatory, anti-inflammatory and antihistaminic activity was included in the formulation.

The oil and certain active ingredients of *N. sativa* especially thymoquinine (TQ) exhibited significant immunomodulatory effect by decreasing the T cell- and natural killer cell-mediated immune responses (Salem, 2005). Strong anti-inflammatory potential of both oil and thymoquinine were also confirmed on various inflammation-based models which were mostly based on the suppression of the prostaglandins and leukotriens inflammatory mediators (Salem, 2005).

Most of the beneficial properties of the *Nigella sativa* seed oil are attributed to TQ. Among its various beneficial effects, the suppression of histamine production and/or release from basophils and

mast cells (Darakhshan et al., 2015) is of high importance in the management of AD. Basic mechanism of its anti-inflammatory activity was trough down-regulation of the expression of pro-inflammatory mediators such as COX-2, iNOS, 5-lipoxygenase, TNF-α and inhibition of the activation of transcription factors NF-κB, Akt and ERK signaling pathways.

Immunomodulatory effects of *N. sativa* is well documented. Following the for 4 weeks intake of *N. sativa* the majority of participants exhibited 55% increase in CD4 to CD8 T cells ratio, and a 30% increase in natural killer (NK) cell function confirming that *N. sativa* can enhance immune responses in humans (Haq et al., 1999). Two times increase in the number of splenic NK cells, and their cytotoxicity against YAC-1 tumor targets when compared to control NK cells was obtained following one week intake of *N. sativa* oil (Abuharfeil et al., 2001). Contrary to its enhancing effect on the T cell-mediated immune response, *N. sativa* constituents have shown a tendency to down-regulate B cell-mediated immunity (Haq et al., 1999). So it could be concluded that *N. sativa* constituent may enhance cellular immunity, while suppress humoral immunity (Salem, 2005).

After the treatment of human peripheral blood mononuclear cells (PBMCs) with methanolic extract of *N. Sativa* with/without previous phytohemagglutinin (PHA) stimulation an immunostimulating effect on non-PHA-stimulated proliferation of human PBMCs was obtained, while in PHA-stimulated cells immunosuppressive activity was found (Alshatwi, 2014).

Nigella sativa reduced significantly the peripheral blood eosinophil count, IgG1 and IgG2a levels, cytokine profiles and inflammatory cells in lung tissue of conalbumin-treated mice, without changing IFN-y level (Abbas et al., 2005) confirming its anti-inflammatory and immunoregulatory effect which may be useful for the treatment of allergic asthma. Pretreatment of the mice with ovalbumin induced allergic airway inflammation with thymoquinine resulted in significant decrease of Th2 cytokines, lung eosinophilia, and goblet cell hyperplasia together with the inhibition of COX-2 protein expression and PGD_2 production confirming a significant anti-inflammatory and immunomodulatory effect of TQ (El Mezayen et al., 2006).

Four weeks treatment of hand eczema patients with *Nigella sativa* oil resulted in significant improvement of the symptoms which was comparable with the results obtained by Betamethasone corticosteroid ointment (Yousefi et al., 2013)

Avena sativa represents the main ingredient in Bioapifit® anti-inflammatory ointment. It was included in the formulation because of its excellent anti-inflammatory, antihistaminic and moisturizing effect (Pazyar et al., 2012). *Avena sativa* was used for centuries for topical treatment of various inflammatory skin diseases. Ilnytska et al., 2016 found that colloidal oatmeal extracts induce the expression of genes related to epidermal differentiation, tight junctions and lipid regulation in skin, and provide pH-buffering capacity. It promotes the expression of multiple target genes related to skin barrier, and resulted in recovery of barrier damage in an *in vitro* model of atopic dermatitis. In *in vivo* study, significant clinical improvements in skin dryness, moisture content, and barrier was obtained following the treatment with colloidal oat based preparation.

Fowler, 2014 summarized the beneficial effect of avenanthramides (the principle polyphenolic antioxidants in oats) for alleviation of the symptoms of AD. Avenanthramides decrease the inflammation in murine models of contact hypersensitivity and neurogenic inflammation as well as pruritogen-induced scratching in a murine itch model. Another study (Cerio et al., 2010) demonstrated that avenanthramides are good inhibitors of nuclear factor kappa B activity resulted with the inhibition of pro-inflammatory cytokines and histamine release from mast cells that play the key role in the pathophysiology of inflammatory dermatoses. As reported by Sur et al., 2008 avenanthramides at concentrations as low as 1 μg/kg inhibited the degradation of nuclear factor kappa B-alpha (NFκBα) inhibitor in keratinocytes which correlated with decreased phosphorylation of p65 subunit of nuclear factor kappa B (NF-κB) as well as tumor necrosis factor-alpha (TNF-α) induced NF-κB luciferase activity resulted in decreased production of IL-8 pro-inflammatory cytokine. Furthermore, avenanthramides also showed anti-itching effect by reducing oxazolone-induced contact hypersensitivity, resiniferatoxin-induced neurogenic inflammation, and compound 48/80-induced, histamine-mediated itch.

Anti-itching effect of oatmeal preparations was demonstrated by Matheson et al., 2001. Topical application of liquid paraffin preparation with 5% colloidal oatmeal significantly decreased itching and need for antihistamine drugs compared to the control group (liquid paraffin only) in the patients with burn injuries.

The treatment of skin fragments stimulated by vasoactive intestinal peptide with oatmeal extract oligomer showed a significant decrease of the mean surface of dilated vessels and edema as well as decrease of TNF-α confirming excellent anti-inflammatory effect of the oligomer (Boisnic et al., 2003).

The colloidal oatmeal extracts decreased pro-inflammatory cytokines production *in vitro* while the application of colloidal oat based skin lotion resulted in significant clinical improvements of skin dryness, scaling, roughness, and itch intensity (Reynertson et al., 2015).

The application of the oat-based sterile emollient cream twice daily in children with AD resulted in significant improvement of clinical symptoms and reduction of the number of flare-ups (Mengeaud et al., 2015).

Criquet et al., 2012 assessed the safety of various oatmeal based personal care products. No allergies were reported by consumers of 445,820 products sold during a 3-year period confirming that colloidal oatmeal is a safe and effective ingredient in personal care products.

The studies conducted on the patients in the age ranging from 3 months to 60 years with mild to moderate atopic dermatitis reviled that the daily use of colloidal oatmeal based moisturizers and/or cleansers resulted in significant decrease of the symptoms. The products were well tolerated in all age groups (Fowler et al., 2012).

Moisturizing effect of colloidal oatmeal is attributed with high content of starches and beta-glucans in oat that are responsible for its protective and water-holding functions (Kurtz and Wallo, 2007).

Bellis perennis was included in the formulation due to its traditional use for the treatment of wounds and bruises. Karakaş et al., 2012 reported excellent wound healing potential of the extract of *B. perennis* flowers on circular excision wound model. Following the 30 days of the treatment with *B. perennis* based ointment significantly higher wound closure was observed compared to the control group. Furthermore, no scars were observed in *B. perennis* treated rats.

It was reported that oleanane-type triterpene saponins isolated from *Bellis perennis* flowers has promoting collagen synthesis in normal human dermal fibroblasts (Morikawa et al., 2015).

Helichrysum italicum was included in the formulation due to its strong anti-inflammatory activity, antimicrobial and wound healing effect (Antunes Viegas et al., 2014). It was reported that acetophenones and flavonoids gnaphaliin and pinocembrin isolated from *H. italicum* could reduce significantly the production of leukotriene B_4 (LTB4) which was resistant to corticosteroid therapy (Ohnishi et al., 2008). LTB4 is pro-inflammatory mediator with chemoattractant properties which is rapidly generated from activated innate immune cells such as neutrophils, macrophages, and mast cells. Elevated levels of leukotriene B4 have been reported in various allergic diseases including AD (Ohnishi et al., 2008). Besides, acetophenones isolated from *H. italicum* exibited the COX-1 activity. Both the whole acetone extract and arzanol isolated from *H. italicum* inhibited NF-κB, while arzanol inhibited the production of IL-1β, TNF-α, IL-6, IL-8 and PGE_2, 5-Lipoxygenase and leukotrienes (Antunes Viegas et al., 2014).

In addition to its excellent anti-inflammatory effect, it was found that *H. italicum* and its constituents posses a strong antimicrobial activity especially against *Staphylococcus aureus* that was often found in AD patients. Nostro et al., 2001 reported excellent inhibitory activity against *S. aureus* both methicillin-resistant and methicillin-sensitive strains in dose-dependent manner. Inhibitory effect was also found in the case of coagulase, DNAse, thermonuclease and lipase enzymes which are considered as virulence factors.

In vivo studies on humans reviled that application of *H. italicum* oil could reduce post-operative scars, local inflammation, edema, bruises and hematomas (Antunes Viegas et al., 2014) that support its traditional use for the treatment of various skin conditions.

Other components are included in the formulations due to its strong wound healing, anti-inflammatory, and antimicrobial activity. Detailed literature data for each constituent concerning *in vitro* and *in vivo* study on animal models are presented in our previous publications (Oreščanin et al., 2015a; Oreščanin et al., 2015b). In addition to that available clinical trials on humans concerning the treatment of various skin conditions will be presented below.

21 patients with 33 venous ulcers were treated twice a day for three weeks with *Calendula officinalis* based ointments. Following the treatment complete epithelialization was achieved in seven patients while the total surface of all the ulcers were reduced for 41.71% compared to only 14.52% in the control group treated with saline solution (Đuran et al., 2005). In the study conducted by Pommier et al., 2004 two nonsteroid topical agents for the prevention of acute dermatitis during adjuvant radiotherapy for breast carcinoma were compared. 126 patients were treated with calendula (Pommade au Calendula par Digestion; Boiron Ltd, Levallois-Perret, France) and other 128 patients with trolamine (Biafine; Genmedix Ltd, France). Significantly lower (P <0.001) occurrence of acute dermatitis of grade 2 or higher was obtained in calendula (41%) compared to the trolamine group (63%). Besides, the patients treated with calendula had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain. The study conducted on 14 male patients (Akhtar et al., 2011) reviled that topical cream containing 3% calendula extract decreased skin erythema significantly confirming its anti-inflammatory effect. Besides, the cream showed skin moisturizing effects and decreased transepidermal water loss.

Nissen et al., 1988 compared 3 different ointments (Kamillosan® ointment, Kamillosan® ointment base and 0.1% hydrocortisone acetate) for the topical treatment of contact dermatitis. The results reviled that Kamillosan® ointment is remarkably superior to other reference products with regard to its soothing effect on human skin. Kamillosan® ointment was found to be about 60% as effective as 0.25% hydrocortisone cream (Albring et al., 1983). Patzelt-Wenczler et al., 2000 reviled that Kamillosan® cream may ease discomfort associated with eczema. In a partially double-blind, randomized study carried out as a half-side comparison, Kamillosan® cream was compared with 0.5% hydrocortisone cream and a placebo consisting only of vehicle cream in patients suffering from medium-degree atopic eczema. After 2 weeks of the treatment, Kamillosan® cream showed a slight superiority over 0.5% hydrocortisone. Glowania et al., 1987 tested the efficacy of the topical use of chamomile extract to enhance wound healing in a double-blind trial on 14 patients who underwent dermabrasion of tattoos. The patients treated with chamomile extracts showed significantly faster wound healing and epithelialization compared to the control. In another randomized, placebo-controlled trial, radiation-induced skin reactions were less frequent and appeared later in chamomile-treated areas, but the differences were not statistically significant (Maiche et al., 1991). Merfort et al., 1994 conducted in vivo skin penetration studies of the chamomile flavones apigenin, luteolin and apigenin 7-O-beta-glucoside with nine healthy, female volunteers. During seven hours the flavonoids are not only adsorbed at the skin surface, but penetrate into deeper skin layers. This is important for their topical use as antiphlogistic agents. In a case study conducted by Carl and Emrich, 1991, 98 cancer patients were used Kamillosan Liquidum® (15 drops in 100 ml of water three times a day), during head and neck irradiation and/or systemic chemotherapy in order to prevent and or reduce the intensity of oral mucositis. Of the 66 patients who participated in prophylactic oral care with the mouthwash, 20 patients underwent radiation therapy and 46 patients received systemic chemotherapy. Only one of the 20 patients who had had radiation therapy developed grade 3 mucositis in the final week of treatment, 65% developed intermediate grade, and 30% developed low-grade mucositis. 36 of 46 patients receiving chemotherapy did not develop clinically significant mucositis. For the 32 patients with existing mucositis, all noted immediate relief from mouth discomfort and within seven days almost all patients returned to having no clinical sign of mucositis. In another clinical trial, 161 patients with eczema on their hands, forearms, and lower legs who had been initially treated with 0.1% difluocortolone valerate were treated with chamomile based Kamillosan® cream, 0.25% hydrocortisone, 0.75% fluocortin butyl ester, or 5% bufexamac. During three to four week maintenance therapy, the Kamillosan® was as effective as 0.25% hydrocortisone. It was superior to 5% bufexamac and 0.75% fluocortin butyl ester (Aertgeerts et al., 1985 a,b)

Vakilian et al., 2011 conducted a randomized control trial on 120 primiparous women with singleton pregnancy who had undergone normal spontaneous vaginal delivery and episiotomy in order to assess the efficiency of lavender oil in wound healing. Experimental group (60 patients) received lavender oil and controls received Povidone-iodine. Incision sites were assessed on the 10th day postpartum. 25 out of 60 women in lavender group and 17 patients in control group had no pain. There was no significant difference between two groups in surgery site complications. However, redness in lavender group was significantly less compared to control group ($p < 0.001$). Similar clinical trial (Sheikhan et al., 2012) included 60 primiparous women admitted for labor in Kamali Hospital in Karaj,

Iran. They were randomly categorized into two groups: experimental (using Lavender oil) and control (using Betadine). Participant's pain and discomfort were recorded using a Visual Analogue Scale (VAS) and a Redness, Edema, Ecchymosis, Discharge Scale (REEDA). Pain was evaluated at 4 h, 12 h and 5 days following episiotomy. There was a statistical difference in pain intensity scores between the two groups after 4 h ($p = 0.002$, and 5 days ($p = 0.000$) after episiotomy. The REEDA score was significantly lower in the experimental group 5 days after episiotomy ($p = 0.000$). According to these findings, use of Lavender oil can be effective in reducing perineal discomfort following episiotomy.

Matić et al., 2009 conducted the study on 39 patients with venous leg ulcers in order to test the ability of the extract of *Achillea millefolium* to accelerate wound healing. The experimental group was treated with an ointment containing 7.5% of yarrow extract for three weeks while for the control group only saline solution dressings were applied to ulcers. Significantly better results were observed in the experimental group with the reduction of total surface area of all ulcers for 39.64% compared to only 15.1% reduction in the control group. The parameters like granulation, epithelization and dermatitis also showed better results in the group treated with yarrow ointment compared to the control.

Similar study (Đuran et al., 1997) was conducted with the Plantoderm ointment containing the extracts of four different medicinal herbs (*Achillea millefolium*, *Calendula officinalis*, *Symphytum officinale*, *Salvia officinalis*). 40 patients with a total number of 66 venous ulcers were treated for three weeks which resulted in 58.55% decrease of total ulcer surface while complete epithelization was recorded in 22 ulcers.

Plantago major leaves extract showed significantly higher antimicrobila potential compared to standard antibiotics (Samuelsen, 2000). Leaves have also traditionally been used for the treatment of skin lesions and for bacterial infections (Holetz et al., 2002). Leaves of *P. major* have been used, and are still being used as a wound healing remedy in almost all parts of the world in folk medicine. Either whole or crushed leaves are used to treat for example burns and other kinds of wounds to enhance the healing process, and to stop bleeding. The leaves of *P. major* have thus been prescribed for the treatment of wounds caused by dog bites (Roca-Garcia, 1972). Normally, it is sufficient to apply only the juice from leaves to heal superficial wounds (Brondegaard, 1987).

Following the treatment of 161 patients with decubitus ulcers with *Symphytum officinale* based cream during four weeks resulted in complete healing of the pressure sores in 85.9 % of the patients and reduction of the total decubitus area for 89.2 % (Stepán et al., 2014). A significant wound healing potential of the topically applied preparation Traumaplant® containing 10% active ingredient from medicinal comfrey was confirmed on the patients with fresh abrasions (Barna et al., 2007; Barna et al., 2012).

4. CONCLUSION

The study included the infants and children with atopic dermatitis in the age range from 4 to 56 months and the onset of the disease between 2 to 6 months. The extent of the affected area of the body ranged from 9 to 82 (37.40±16.95), intensity of the objective symptoms from 5 to 18 (12.92±.4.40), subjective symptoms from 2 to 20 (13.80±5.55) and total score from 23.1 to 99.4 (66.50±23.43). Among the tested patients 76% of them developed severe symptoms of AD, 20% of them had moderate and only 4% them had mild symptoms. There was a strong, statistically significant influence of the predictor variables on the total score ($R = 0.89$; $p< 0.00000$) and their influence increased in the following order: other allergic diseases<age<family history. Following the eight weeks of the treatment the extent, intensity and subjective symptoms decreased significantly. The total score decreased more than 20 times compared to the period before the therapy and ranged from 0 to 14 (13.18±4.29). The intensity parameters (swelling, oozing/crusting and scratch marks) as well as subjective parameters (itch and sleeplessness) disappeared completely following two months of the therapy. Two months of the topical treatment with Bioapifit® herbal anti-inflammatory ointment resulted in complete remission of the symptoms in 56% of the patients while in another 44% of them only mild symptoms are recorded. Such excellent results could be ascribed to the ointment's formulation containing emollients, strong anti-inflammatory, immunomodulating, wound healing and antimicrobial agents of herbal origin that simultaneously targeted the multiple mechanisms involved in

AD pathogenesis. This ointment could be used as alternative therapy to topical corticosteroids and immunomodulants.

5. REFERENCES:

1. Abbas AT, Abdel-Aziz MM, Zalata KR, Abd Al-Galel Tel-D. Effect of dexamethasone and *Nigella sativa* on peripheral blood eosinophil count, IgG1 and IgG2a, cytokine profiles and lung inflammation in murine model of allergic asthma. Egypt J Immunol 2005;12(1):95-102.
2. Abuharfeil NM, Salim M, Von Kleist S. Augmentation of natural killer cell activity in vivo against tumour cells by some wild plants from Jordan. Phytother Res 2001;15:109–113.
3. Aertgeerts P, Albring M, Klaschka F, Nasemann T, Patzelt-Wenczler R, Rauhut K, Weigl B. Comparative testing of Kamillosan cream and steroidal (0.25% hydrocortisone, 0.75% fluocortin butyl ester) and non-steroidal (5% bufexamac) dermatologic agents in maintenance therapy of eczematous diseases. Z Hautkr 1985a;60(3):270-277.
4. Aertgeerts P, Albring M, Klaschka F, Patzelt-Wenczler R, Rauhut K, Weigl B. Comparison of Kamillosan (TM) cream (2 g ethanolic extract from chamomile flowers in 100 g cream) versus steroidal (0.25% hydrocortisone, 0.75% fluocortin butyl ester) and non-steroidal (5% bufexamac) dermatics in the maintenance therapy of eczema. Z Hautkr 1985b;60:270-277.
5. Akhtar N, Zaman S, Khan BA, M H, Khan S, Ahmad M, Rasool F, Mahmood T, Rasul, A. Evaluation of various functional skin parameters using a topical cream of *Calendula officinalis* extract. Afr J Pharm Pharmacol 2011;5(2):199-206.
6. Albring M, Albrecht H, Alcorn G, Lüker, PW. The measuring of the anti-inflammatory effect of a compound on the skin of volunteers. Methods Find Exp Clin Pharmacol 1983;5:75-77.
7. Allen KJ, Dharmage SC. The role of food allergy in the atopic march. Clin Exp Allergy 2010;40:1439-1441.
8. Alshatwi AA. Bioactivity-guided identification to delineate the immunomodulatory effects of methanolic extract of Nigella sativa seed on human peripheral blood mononuclear cells. Chin J Integr Med 2014, DOI:10.1007/s11655-013-1534-3
9. Antunes Viegas D, Palmeira-de-Oliveira A, Salgueiro L, Martinez-de-Oliveira J, Palmeira-de-Oliveira R. Helichrysum italicum: from traditional use to scientific data. J Ethnopharmacol 2014;151(1):54-65.
10. Barna M, Kucera A, Hladíkova M, Kucera, M. Randomized double-blind study: wound-healing effects of a Symphytum herb extract cream (Symphytum×uplandicum Nyman) in children. Arzneimittelforschung 2012;62(6):285-289.
11. Barna M, Kucera A, Hladícova M, Kucera, M. Wound healing effects of a *Symphytum* herb extract cream (*Symphytum* x uplandicum NYMAN): results of a randomized, controlled double-blind study. Wien Med Wochenschr 2007;157(21-22):569-574.
12. Bieber T. Atopic dermatitis. Ann Dermatol 2010;22(2):125-137.
13. Bieber T. Atopic dermatitis. N Engl J Med 2008;358(14):1483-1494.
14. Bohme W, Wickman M, Lennart Nordvall S, Svartengren M, Wahlgren CF. Family history and risk of atopic dermatitis in children up to 4 years. Clin Exp Allergy 2003;33(9):1226-1231.
15. Boisnic S, Branchet-Gumila MC, Coutanceau C. Inhibitory effect of oatmeal extract oligomer on vasoactive intestinal peptide-induced inflammation in surviving human skin. Int J Tissue React 2003;25(2):41-46.
16. Boucetta K Q, Charrouf Z, Aguenaou H, Derouiche A, Bensouda Y. The effect of dietary and/or cosmetic argan oil on postmenopausal skin elasticity. Clin Interv Aging 2015; 10: 339–349.
17. Boucetta K Q, Charrouf Z, Derouiche A, Rahali Y, Bensouda Y. Skin hydration in postmenopausal women: argan oil benefit with oral and/or topical use. Prz Menopauzalny 2014; 13(5): 280–288.
18. Brandt EB, Sivaprasad U. Th2 Cytokines and Atopic Dermatitis. J Clin Cell Immunol 2011;2(3): doi:10.4172/2155-9899.1000110.
19. Brondegaard, VJ. Folk of flora. Rosenkilde Bagger, Kobenhavn, 1987, pp. 68–77.
20. Carl W, Emrich, LS. Management of oral mucositis during local radiation and systemic chemotherapy: a study of 98 patients. J Prosthet Dent 1991;66(3):361-369.

21. Carlsten C, Dimich-Ward H, Ferguson A, Watson W, Rousseau R, Dybuncio A, et al. Atopic dermatitis in a high-risk cohort: natural history, associated allergic outcomes, and risk factors. Ann Allergy Asthma Immunol 2013;110(1):24-28.
22. Carson CG. Risk factors for developing atopic dermatitis. Dan Med J 2013;60(7):B4487.
23. Centerchem Inc. Sweet Almond oil, V03-0/06; http://www.centerchem.com/Products/DownloadFile.aspx?FileID=6849
24. Cerio R, Dohil M, Jeanine D, Magina S, Mahé E, Stratigos AJ. Mechanism of action and clinical benefits of colloidal oatmeal for dermatologic practice. J Drugs Dermatol 2010;9:1116-1120.
25. Criquet M, Roure R, Dayan L, Nollent V, Bertin C. Safety and efficacy of personal care products containing colloidal oatmeal. Clin Cosmet Investig Dermatol 2012;5:183-193.
26. Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res 2015;95-96:138-158.
27. Deckers IA, McLean S, Linssen S, Mommers M, van Schayck CP, Sheikh A. Investigating international time trends in the incidence and prevalence of atopic eczema 1990-2010: a systematic revieww of epidemiological studies. PLoS ONE 2012;7:e39803.
28. Dharmage SC, Lowe AJ, Matheson MC, Burgess JA, Allen KJ, Abramson MJ. Atopic dermatitis and the atopic march revisited. Allergy 2014;69:17-27.
29. Đuran V, Matić M, Jovanovć M, Mimica N, Gajinov Z, Poljački M, Boza, P. Results of the clinical examination of an ointment with marigold (*Calendula officinalis*) extract in the treatment of venous leg ulcers. Int J Tissue React 2005;27(3):101-106.
30. Đuran V, Matić M, Zrnić B, Poljački M, Jovanović M, Dimoski A. Rezultati kliničkog ispitivanja Plantoderm masti u bolesnika sa venskim ulceracijama donjih ekstremiteta. Arh Farm 1997;5: 526-527.
31. El Mezayen R1, El Gazzar M, Nicolls MR, Marecki JC, Dreskin SC, Nomiyama H. Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol Lett 2006;106(1):72-81.
32. Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. Allergy 2014;69:3-16.
33. Fowler JF. Colloidal oatmeal formulations and the treatment of atopic dermatitis. J Drugs Dermatol 2014;13(10):1180-1183.
34. Fowler JF, Nebus J, Wallo W, Eichenfield LF. Colloidal oatmeal formulations as adjunct treatments in atopic dermatitis. J Drugs Dermatol 2012;11(7):804-807.
35. Glowania HJ, Raulin C, Swoboda M. Effect of chamomile on wound healing-a clinical double-blind study. Z Hautkr 1987;62:1267–1271.
36. Gustafsson D, Sjöberg O, Foucard T. Development of allergies and asthma in infants and young children with atopic dermatitis-a prospective follow-up to 7 years of age. Allergy 2000;55:240-245.
37. Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. Int J Immunopharmacol 1999;21:283–295.
38. Hayakawa R, Ueda H, Nozaki T, Izawa Y, Yokotake J, Yazaki K, Azumi T, Okada Y, Kobayashi M, Usuda T, Ishida J, Kondo T, Adachi A, Kawase A, Matsunaga K. Effects of combination treatment with vitamins E and C on chloasma and pigmented contact dermatitis. A double blind controlled clinical trial. Acta Vitaminol Enzymol 1981;3(1):31-38.
39. Hershko AY, Suzuki R, Charles N, Alvarez-Errico D, Sargent JL, Laurence A, et al. Mast cell interleukin-2 production contributes to suppression of chronic allergic dermatitis. Immunity 2011;35(4):562-571.
40. Holetz FB, Pessini GI, Sanches NR, Cortez DA, Nakamura CV, Filho, BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 2002;97(7):1027-1031.
41. Hvid M, Vestergaard C, Kemp K, Christensen GB, Deleuran B, Deleuran M. IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction?. J Invest Dermatol 2011;131(1):150-157.

42. Ilnytska O, Kaur S, Chon S, Reynertson KA, Nebus J, Garay M, Mahmood K, Southall MD. Colloidal Oatmeal (Avena Sativa) Improves Skin Barrier Through Multi-Therapy Activity. J Drugs Dermatol 2016;15(6):684-690.
43. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, Nakanishi K, Yamanishia K. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. Proc Natl Acad Sci U S A. 2013;110(34):13921-13926.
44. ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjuctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006;368:733-743.
45. Javanbakht MH, Keshavarz SA, Djalali M Siassi F, Eshraghian MR, Firooz A, Seirafi H, Ehsani AH, Chamari M, Mirshafiey A. Randomized controlled trial using vitamins E and D supplementation in atopic dermatitis. J Dermatolog Treat 2011;22(3):144-150.
46. Karakaş FP, Karakaş A, Boran Ç, Türker AU, Yalçin FN, Bilensoy E. The evaluation of topical administration of Bellis perennis fraction on circular excision wound healing in Wistar albino rats. Pharm Biol 2012;50(8):1031-1037.
47. Kelleher M, Dunn-Galvin A, Hourihane J O'B, Murray D, Campbell LE, McLean WHI, Irvine AD. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. J Allergy Clin Immunol 2015;135(4):930-935.
48. Keller KL, Fenske NA. Uses of vitamins A, C, and E and related compounds in dermatology: a review. J Am Acad Dermatol 1998;39:611-625.
49. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, Hepworth MR, Van Voorhees AS, Comeau MR, Artis D. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. Sci Transl Med 2013 5 (170):170.
50. Kim BS, Wang K, Siracusa MC, Saenz SA, Brestoff JR, Monticelli LA, et al. Basophils promote innate lymphoid cell responses in inflamed skin. J Immunol 2014;193(7):3717-3725.
51. Kim BS. Innate lymphoid cells in the skin. J Invest Dermatol 2015;135(3):673-678.
52. Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. J Invest Dermatol 2008;128(11):2625-2630.
53. Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. J Clin Invest 2012;122(2):440-447.
54. Kurtz ES, Wallo W. Colloidal oatmeal: History, chemistry and clinical properties. J Drugs Dermatol 2007;6:167-170.
55. Lavagna SM, Secci D, Chimenti P, Bonsignore L, Ottaviani A, Bizzarri, B. Efficacy of Hypericum and Calendula oils in the epithelial reconstruction of surgical wounds in childbirth with caesarean section. Farmaco 2001;56:451–453.
56. Leung TNH, Hon KL. Eczema therapeutics in children: what do the clinical trials say? Hong Kong. Med J 2015;21:251–260.
57. Maiche A, Grohn P, Maki-Hokkonen, H. Effect of chamomile cream and almond ointment on acute radiation skin reaction. Acta Oncol 1991;30:395-396.
58. Margolis DJ, Kim B, Apter AJ, Gupta J, Hoffstad O, Papadopoulos M, Mitra N. Thymic stromal lymphopoietin variation, filaggrin loss of function, and the persistence of atopic dermatitis. JAMA Dermatol 2014;150(3):254-259.
59. Matić M, Đuran V, Jovanović M, Gajinov Z, Matić A, Đuran B, Pal B, Mimica-Dukić, N. Treatment of venous leg ulcers with an ointment containing yarrow (*Achillea millefolium*) extract. Serb J Dermatol Venereol 2009;3:97-136.
60. Matheson JD, Clayton J, Muller MJ. The reduction of itch during burn wound healing. J Burn Care Rehabil 2001;22:76-81.
61. Mengeaud V, Phulpin C, Bacquey A, Boralevi F, Schmitt AM, Taieb A. An innovative oat-based sterile emollient cream in the maintenance therapy of childhood atopic dermatitis. Pediatr Dermatol 2015;32(2):208-215.
62. Merfort I, Heilmann J, Hagedorn-Leweke U, Lippold BC. In vivo skin penetration studies of camomile flavones. Pharmazie 1994;49(7):509-511.
63. Molfino NA, Gossage D, Kolbeck R, Parker JM, Geba GP. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. Clin Exp Allergy 2012;42(5):712-737.

64. Morikawa T, Ninomiya K, Takamori Y, Nishida E, Yasue M, Hayakawa T, Muraoka O, Li X, Nakamura S, Yoshikawa M, Matsuda H. Oleanane-type triterpene saponins with collagen synthesis-promoting activity from the flowers of Bellis perennis. Phytochemistry 2015;116:203-212.
65. Nicolaou A. Eicosanoids in skin inflammation. Prostaglandins Leukot Essent Fatty Acids 2013;88(1):131-138.
66. Nissen HP, Blitz H, Kreyel HW. Prolifometrie, eine methode zur beurteilung der therapeutischen wirsamkeit kon Kamillosan®-Salbe. Z Hautkr 1988;63:84–90.
67. Nostro A, Bisignano G, Angela Cannatelli M, Crisafi G, Paola Germanò M, Alonzo V. Effects of Helichrysum italicum extract on growth and enzymatic activity of Staphylococcus aureus. Int J Antimicrob Agents 2001;17(6):517-520.
68. Ohnishi H, Miyahara N, Gelfand EW. The Role of Leukotriene B4 in Allergic Diseases. Allergol Int 2008;57:291-298.
69. Oranje AP. Practical issues on interpretation of scoring atopic dermatitis: SCORAD Index, objective SCORAD, patient-oriented SCORAD and Three-Item Severity score. Curr Probl Dermatol 2011;41:149-155.
70. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 2007;157(4):645-648.
71. Oreščanin V, Findri Guštek Š, Krivak Bolanča I. Development and Application of New Herbal Pessaries for the Treatment of Squamous Endocervical Metaplasia. Ind J App Res 2015a:5(6),176-182.
72. Oreščanin V, Findri Guštek Š, Hunjak, B. Application of new Herbal Pessaries for the Treatment of the Lower Genital Tract Infections. Ind J App Res 2015b:5(6), 510-516.
73. Oreščanin V, Findri Guštek Š. Development And Testing of New Herbal Ointment for the Treatment of Stress Urinary Incontinence-A Preliminary Study. Global J Res Anal 1016;5(7):371-374.
74. Osawa R, Akiyama M, Shimizu H. Filaggrin gene defects and the risk of developing allergic disorders. Allergol Int 2011; 60(1):1-9.
75. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, Goudie DR, Sandilands A, Campbell LE, Smith FJ, O'Regan GM, Watson RM, Cecil JE, Bale SJ, Compton JG, DiGiovanna JJ, Fleckman P, Lewis-Jones S, Arseculeratne G, Sergeant A, Munro CS, El Houate B, McElreavey K, Halkjaer LB, Bisgaard H, Mukhopadhyay S, McLean WH. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006;38(4):441-446.
76. Patzelt-Wenczler R, Ponce-Pöschl, E. (2000). Proof of efficacy of Kamillosan®cream in atopic eczema. Eur J Med Res 2000;5(4):171-175.
77. Pazyar N, Yaghoobi R, Kazerouni A, Feily A. Oatmeal in dermatology: a brief review. Indian J Dermatol Venereol Leprol 2012;78(2):142-145.
78. Peng W, Novak N. Pathogenesis of atopic dermatitis. Clin Exp Allergy 2015;45(3):566-74.
79. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, Montbarbon, X. Phase III randomized trial of *Calenduala Officinalis* compared with Tromaline for the prevention of acute dermatitis during irradiation for breast cancer. J Clin Oncol 2004;22(8):1447-1453.
80. Pustisek N. The impact of structured education on the clinical course of atopic dermatitis. PhD thesis, Univeristy of Zagreb, 2016, p. 117
81. Reynertson KA, Garay M, Nebus J, Chon S, Kaur S, Mahmood K, Kizoulis M, Southall MD. Anti-inflammatory activities of colloidal oatmeal (*Avena sativa*) contribute to the effectiveness of oats in treatment of itch associated with dry, irritated skin. J Drugs Dermatol 2015;14(1):43-48.
82. Roediger B, Kyle R, Yip KH, Sumaria N, Guy TV, Kim BS, et al. Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells. Nat Immunol 2013;14(6):564-573.
83. Roca-Garcia, H. Weeds: a link with the past. Arnoldia 1972;30:23–24.
84. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol 2005;5(13-14):1749-1770.

85. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, Huang L-C, Johnson D, Scanlon ST. McKenzie ANJ, Fallon PG, Ogg GS. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. J Exp Med 2013;210(13):2939-2950.
86. Samuelsen AB, Lund I, Djahromi JM, Paulsen BS, Wold JK, Knutsen, SH. Structural features and anti-complementry activity of some heteroxylan polysaccharide fractions from the seeds of *Plantago major* L. Carb Pol 1999;38:133–143.
87. Samuelsen, AB. The traditional uses, chemical constituents and biological activities of Plantago major L. A review. J Ethnopharmacol 2000;71:1–21.
88. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimäki S, et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. J Invest Dermatol 2012;132(5):1392-1400.
89. Schmitt J, Langan S, Deckert S, Svensson A, von Kobyletzki L, Thomas K, Spuls P. Assessment of clinical signs of atopic dermatitis: a systematic review and recommendation. J Allergy Clin Immunol 2013;132(6):1337-1347.
90. Smith FJ, Irvine AD, Terron-Kwiatkowski A, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006;38(3):337-342.
91. Spergel JM. From atopic dermatitis to asthma: the atopic march. Ann Allergy Asthma Immunol 2010;105(2):99-106.
92. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, Liu YJ. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002;3(7):673-680.
93. Stepán J, Ehrlichova J, Hladikova, M. Efficacy and safety of symphytum herb extract cream in the treatment of pressure ulcers. Z Gerontol Geriatr 2014;47(3):228 -235.
94. Sur R, Nigam A, Grote D, Liebel F, Southall MD. Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. Arch Dermatol Res 2008;300:569-574.
95. Tichota DM, Silva AC, Sousa Lobo JM, Amaral MH. Design, characterization, and clinical evaluation of argan oil nanostructured lipid carriers to improve skin hydration. Int J Nanomedicine 2014; 9:3855–3864.
96. Tsoureli-Nikita E, Hercogova J, Lotti T, Menchini G. Evaluation of dietary intake of vitamin E in the treatment of atopic dermatitis: a study of the clinical course and evaluation of the immunoglobulin E serum levels. Int J Dermatol 2002;41(3):146-150.
97. Vakilian K, Atarha M, Bekhradi R, Chaman, R. Healing advantages of lavender essential oil during episiotomy recovery: A clinical trial Complement Ther Clin Pract. 2011;17(1):50 -53.
98. Yousefi M, Barikbin B, Kamalinejad M, Abolhasani E, Ebadi A, Younespour S, Manouchehrian M, Hejazi S. Comparison of therapeutic effect of topical Nigella with Betamethasone and Eucerin in hand eczema. J Eur Acad Dermatol Venereol 2013;27(12):1498-504.