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Andrographolide a New Potential Drug for the Long Term Treatment of Rheumatoid Arthritis Disease

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Additional information is available at the end of the chapter

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1. Introduction

Andrographis paniculata, (Burm. f.) Wall. ex Nees, a herbaceous plant belonging to the Family Acanthaceae, is one of the most commonly used medicinal plants in the traditional systems of Unani and Ayurvedic medicines. It grows in hedge rows throughout the plains of India and is also cultivated in gardens. It also grows in many other Asian countries and is used as a traditional herbal medicine in China, Hong Kong, the Philippines, Malaysia, Indonesia, and Thailand. It is an annual plant of 1-3 ft high, also known as the “king of bitters”, being the aerial parts most commonly used. *A. paniculata* have shown a broad range of pharmacological effects such as inhibition of replication of the HIV virus, prevention of common cold, and antimalarial, antidiarrheal, antibacterial, antihyperglycemic effects, suppression of various cancer cells, and principally anti-inflammatory properties. Andrographolide is the major labdane diterpenoid isolated from *A. paniculata* and exhibits anti-inflammatory and anticancer activities, either *in vitro* or *in vivo* experimental models of inflammation and cancer. Several immunomodulatory responses of andrographolide have been observed in *in vitro* studies, such as reduction of iNOS, COX-2, NO, PGE2, TNF- α and IL-12 in macrophages and microglia. In neutrophils is able to reduce the radical oxygen species production, and Mac-1, IL-8 and COX-2 expression. In T cells, andrographolide inhibits the expression of IL-2, IFN γ and IL-6, reducing the humoral and cellular adaptive immune response. Andrographolide was able to reduce the dendritic cells maturation and their ability to present antigens to T cells. Andrographolide administered in rodents reduced the Th2 cytokine IL-4, IL-5, IL-13 and serum immunoglobulin in an ovalbumin induced asthma model. A reduction of T cells response also has been observed in experimental autoimmune encephalomyelitis and systemic lupus erythematosus mouse model. Several of immunomodulatory responses have been associated to the inhibition of Nuclear Factor- κ B

functions. It has been demonstrated that andrographolide inhibits the nuclear translocation of the p65 subunit of NF- κ B and interferes with the NF- κ B binding to the DNA. Also andrographolide can reduce NFAT function in T cells and reduce the phosphorylation of signal transducer and activator of transcription-3 (STAT3) in macrophages.

We propose the potential use of andrographolide in Rheumatoid Arthritis and other autoimmune diseases. This is supported by the fact that andrographolide exerts anti-growth and proapoptotic effects on human rheumatoid arthritis fibroblast-like synoviocytes, the main cellular constituent of pannus, that combined with a massive infiltration of lymphocytes and macrophages, invades and destroys the local articular structure. Recently, a prospective randomized placebo-controlled trial has suggested that *A. paniculata*, a standardized extract containing NLT 30% of andrographolide was effective for symptom relief in patients with Rheumatoid Arthritis. The use of andrographolide alone or a patented *A. paniculata* standardized extract in clinical trials shows mild and few side effects, and has the potential to be developed into a new alternative drug for Rheumatoid Arthritis treatment in the long term.

2. *Andrographis paniculata* and labdane diterpenoids

The main and most interesting biological constituent of *A. paniculata* herb (aerial part) is a group of diterpene lactones belonging to the ent-labdane class, present in both free and glycosidic forms, and named andrographolides [1, 2].

Andrographolide is the bitter principle, a colourless, neutral crystalline substance, was first isolated by Boorsma from different parts of *Andrographis paniculata* [2]. In 1911 Gorter proved that it is structurally a lactone and named it andrographolide (in the Chinese literature it is sometimes cited as andrographis B). The bitter principle has been subjected to a number of chemical investigations. The properties of the compound and its diterpenoid lactone nature, as well as its stereochemistry, conformation and crystal structure were cleared by means of infrared, x, mass spectrometry and NMR analysis. Its chemical formula corresponds to the 3,14,15,18-tetrahydroxy-5,9 H,10-labda-8(20),12-dien-16-oic acid-lactone (Figure 1). Most recently, various epimers, geometric isomers, and rearrangement products of andrographolide have been isolated and structurally characterized [3, 4]. Andrographolide, as the other diterpene lactones of *A. paniculata*, are generally extracted with CHCl₃/EtOH or acetone, and several methods are described in the literature to determinate its content in the plant, in commercial formulation, i.e. standardized extract and in biological samples: titration with alkalis, TLC/UV spectrophotometry and HPLC methods. The maximum content of andrographolide and related diterpenoids is in the mature leaves. It has been described that the stem contained 0.2±0.02%, seeds 0.13±0.01%; root 0.44±0.01%; and leaves 2.39±0.008% of andrographolide [5]. Regional variation in the andrographolide content was also observed. The content of andrographolide varies with the harvest season. The leaves contain more than 2% andrographolide before the plant blossoms; afterward the contents decreases to less than 0.5% [2]. The pH modifies the stability of andrographolide, and hydrolysis is extremely slow below pH 7, but considerably faster on the alkaline side, producing some structural changes. Androgra-

pholide is sparingly soluble in water; soluble in acetone, methanol, chloroform and ether. As a water soluble andrographolide derivative, the sodium bisulfite adduct has been synthesized for medical use as an antipyretic agent.

Preclinical properties include anti-retroviral [6, 7], antiproliferative and pro-apoptotic [8, 9], anti-diabetic [10, 11], anti-angiogenic [12], anti-thrombotic [13], anti-urothelial [14], anti-leishmaniasis [15], hepatoprotective [16, 17], protective activity against alcohol-induced hepatic and renal toxicity [18], and cardioprotective [19] and anti-inflammatory [20-25] properties.

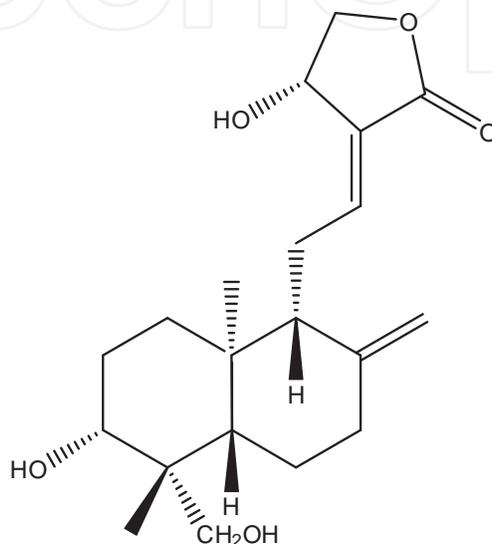


Figure 1. Chemical structure of andrographolide

2.1. Neoandrographolide

The second diterpene isolated from *A. paniculata* was the minor non-bitter constituent neoandrographolide, which was first described by Kleipool in 1952. The structure of neoandrographolide (Figure 2) was described as a diterpene glucoside and its amount in the plant is around 0.5-1%. The main preclinical effects are anti-inflammatory [23, 26, 27], chemosensitizer [28], anti-herpes-simplex virus [7] and antioxidant [29].

2.2. Minor labdane diterpenes

Afterwards, more than 20 other diterpene lactones, both glycosylated and not, have been described. The most important among them, characterized by Balmain and Connolly in 1973, are: 14-deoxy-11,12- didehydroandrographolide, with an average content in the leaf of 0.1%, 14-deoxyandrographolide (0.02%), 14-deoxy-11-oxoandrographolide (0.12%) (Figure 2) [3]. In other hand has been described that 14-deoxy-11,12- didehydroandrographolide possess vasorelaxant and antihypertensive [30, 31], anti-herpes [7], antioxidant and hepatoprotective [32], antithrombotic [33], antiretroviral [6], and antidiabetic properties [34]. Meanwhile 14-deoxyandrographolide exert hepatoprotective [35], uterine smooth muscle relaxant [36],

immunomodulator [37], platelet activating factor antagonist [38], and vasorelaxant and antihypertensive [39] effects. In addition, 14-deoxy-11-oxoandrographolide only has been reported antileishmaniasis effect [40].

Andrographiside, the 19-glucoside of andrographolide, was isolated in 1981, and only a hepatoprotective effect has been described [41].

A. paniculata contains also minor andrographolide-like compounds such as andropanoside (19-glucoside of 14-deoxy-andrographolide), or andrograpanin (3,14-dideoxy-andrographolide), which are mostly all 14-deoxy- and/or 3-deoxy-derivatives. These compounds show anti-inflammatory properties in preclinical studies [42, 43].

Isoandrographolide is present in the whole plants and has been described as a cellular differentiation inducer [3], antiproliferative [44], and cytotoxic [45] effects.

Also three salts of labdanic acids, named as magnesium andrographate, disodium andrographate and dipotassium andrographate 19-O-D-glucoside have been isolated hydrophylic extract from the leaf.

Since the total synthesis of andrographolide and analogues, many libraries of new derivatives have been created using andrographolide as a template with the purpose to obtain compounds with improved pharmacological profiles. Andrographolide is also a starting point for the semisynthesis of other labdane diterpenes [46-48].

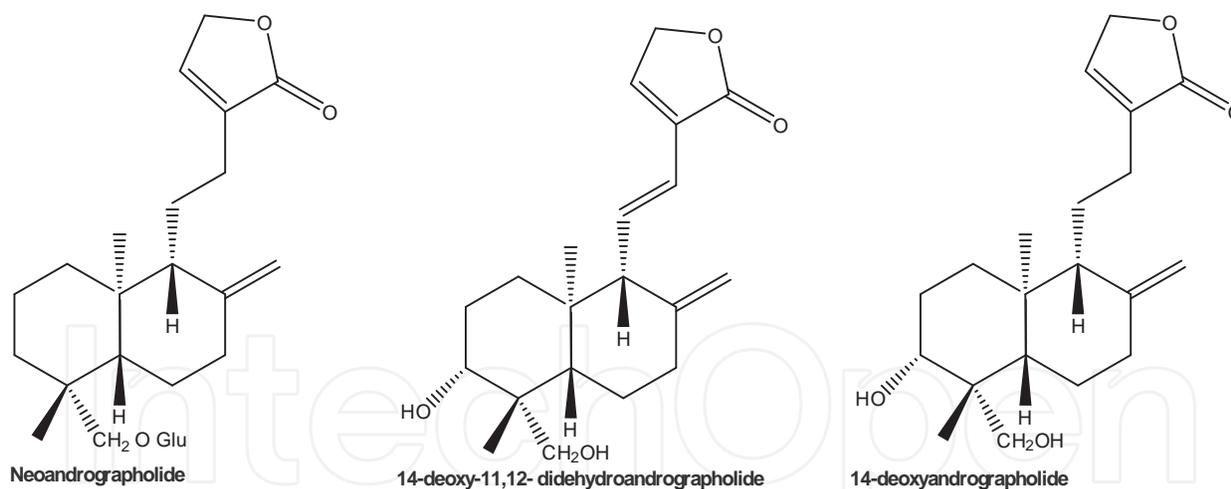


Figure 2. Chemical structure of minor labdane diterpenes isolated from *Andrographis paniculata*.

3. Anti-inflammatory and immunomodulatory effects of andrographolide *in vitro* and *in vivo*

Different preparations of *A. paniculata* administered orally reduced the pyrexia within or after 5 hrs of administration of yeast in rats [49]. On the other hand, administration of *A. panicula-*

ta (20 mg/100 g b.w.) one hour before the injection of carrageenin, reduced the edema in 65.3% in rats. The effect was comparable to oxyphenilbutazone 76.5% [2]

3.1. *In vitro* studies

Andrographolide, shows anti-inflammatory and anticancer activities in both *in vitro* and *in vivo*. The effects of andrographolide on two cells types that play an important role in the inflammatory processes, e.g. leukocyte (neutrophils, macrophages and T-cells) and endothelial cells, demonstrates the ability of this compound to reduce the expression and production of pro-inflammatory mediators.

Several *in vitro* studies show that andrographolide reduces the production of the oxygen radical superoxide anion and hydrogen peroxide, as well as the adhesion induced by chemoattractant in isolated neutrophils [50, 51]. Other antecedents describe a reduction of the expression of cyclooxygenase-2 (COX-2), inducible enzyme producing prostaglandins, in a human model of neutrophils [21]. In mouse peritoneal macrophages, andrographolide reduces the production stimulated by lipopolysaccharide (LPS) of two important cytokines that participate in the amplification and activation of the inflammatory process, the cytokines tumoral necrosis factor TNF α and granulocyte macrophage colony-stimulating factor (GM-CSF). The inhibition of the release of these cytokines by andrographolide was compared to the synthetic glucocorticoid dexamethasone, showing andrographolide to have a similar effect as dexamethasone, but with a lower potency [24, 52]. Also, the effect of andrographolide on the cellular chemotaxis, a response that allows the movement of inflammatory cells to the injured tissue, show that it reduces the chemotactic migration of macrophage induced by C5a, which may contribute to its anti-inflammatory activity [53]. In local or systemic inflammatory disorders there is an enhanced formation of nitric oxide (NO) following the expression of inducible nitric oxide synthase (iNOS). The inhibition of NO formation may have therapeutic benefit in patients with inflammatory diseases as Rheumatoid Arthritis [54]. Thus, andrographolide reduces the LPS-induced iNOS and COX-2 expression in RAW264.7 macrophages [55, 56]. Additionally, andrographolide may have an effect on inflammation-mediated neurodegeneration, since it reduces the production of reactive oxygen species (ROS), TNF α , NO and prostaglandin E2 in microglia, the counterpart of macrophages in the brain [25]. Andrographolide reduces the *in vitro* activation of human and murine T-cells, T-cells proliferation, interleukin-2 (IL-2) and IFN γ production [57-60].

Interaction of leukocyte-endothelium plays a key role in the initiation and maintenance of inflammation, being the adhesion molecule ICAM-1 important in mediating leukocyte adhesion, arrest and transmigration to the inflammatory site. In this respect, certain antecedents show that andrographolide reduces the adhesion of HL-60 cells onto human vein endothelial cells (HUVEC) and the expression of TNF α -induced ICAM-1 [61, 62]. In addition, andrographolide reduces the endothelial cell proliferation, migration and invasion, suggesting a role in angiogenesis [63]. Moreover, andrographolide reduces the growth factor deprivation-induced apoptosis in endothelial cells [64].

The therapeutic potential of andrographolide for the treatment of rheumatoid arthritis has been suggested by using of human rheumatoid arthritis fibroblast-like synoviocytes (RAFLSs) as a cellular model. Andrographolide exerts anti-proliferative and pro-apoptotic effects in RAFLSs, with G0/G1 cell cycle arrest, increases the expression of cell-cycle inhibitors p21 and p27 and reduces cyclin-dependent kinase 4 [65].

3.2. *In vivo* studies

The anti-inflammatory activity of andrographolide has been studied in diverse *in vivo* inflammatory diseases models.

Earlier studies with andrographolide show that it inhibited carrageenin, kaolin and nistatin-induced paw oedema. Moreover, andrographolide p.o. significantly inhibited the weight of granuloma induced by cotton pellets, and decreased the edema in adjuvant-induced arthritis (0.1-0.4% dead *Mycobacterium tuberculosis* suspension). Andrographolide (300 mg/kg) also inhibited dye leakage in acetic acid-induced vascular permeability. It was devoided of any ulcerogenic effect on the stomach in acute and chronic studies in rats. These effects were dose dependent, but inferior to phenylbutazone. Other diterpenic lactones, have shown to possess antipyretic effect in rabbits and rats with fever induced by 2-4-dinitrophenol. The potency was: 14-deoxy-11,12-didehydroandrographolide > deoxyandrographolide, and neoandrographolide > andrographolide [66].

In a model of ovalbumin-induced asthma in mice the intra-peritoneal administration of 30 mg/kg andrographolide reduces the levels of TNF α and GM-CSF (92 and 65 %, respectively) in bronchoalveolar fluid, and the accumulation of lymphocytes and eosinophils, supporting a potential use in asthma. Andrographolide also reduced the Th2 cytokine IL-4, IL-5, IL-13 and serum immunoglobulin [20, 52].

Andrographolide also is helpfulness in the reduction of the symptoms of a mice experimental autoimmune encephalomyelitis (EAE), an animal model of human Multiple Sclerosis, by inhibiting T-cell and antibody responses directed to myelin antigens [59]. Similarly, in another model of autoimmune disease, the administration of andrographolide reduces the susceptibility, prevents the symptoms and reduces anti-nuclear antibodies and kidney damage of systemic lupus erythematosus [67, 68].

The potential effect of andrographolide on rheumatoid arthritis could involve angiogenesis inhibition. In fact, the development of new vessels, is important process that might facilitate the incoming of inflammatory cells into the synovium and, therefore, stimulate the pannus formation. [69]. In a model of induction of angiogenesis in C57BL/6 mice, andrographolide reduced the serum levels of cytokines of IL-1 β , IL-6, TNF α and GM-CSF, the angiogenic factor VEGF and the NO production. Additionally, it is observable an increase of the levels of anti-angiogenic factors TIMP-1 and IL-2 [12]. Andrographolide also suppresses breast tumor growth, which correlates with the inhibition of the pro-angiogenic molecules OPN and VEGF, in the NOD/SCID mice model [70].

4. Anti-inflammatory molecular mechanisms of andrographolide

All immunomodulatory effects of andrographolide have been attributed to modulation of different intracellular mediators, however three main mechanisms are commonly described. A first anti-inflammatory mechanism involved in the reduction of COX-2 expression by andrographolide in neutrophils comprises the modulation of the NF- κ B pathway. The NF- κ B is a family of transcription factors that regulate the expression of a large number of pro-inflammatory genes, such as COX-2, iNOS, TNF- α , IL-8 or IL-1, that are involved in the pathogenesis of Rheumatoid Arthritis. The activation of NF- κ B comprises two main routes: the canonical and alternative pathways. The canonical NF- κ B signaling pathway is the most important one. Inflammatory receptor activation results in I κ B kinase (IKK) activation, and the IKK complex phosphorylate the I κ B protein, leading to its polyubiquitination. The ubiquitinated I κ B is degraded via 26S proteasome, thereby exposing the nuclear localization signal on NF- κ B dimer and inducing nuclear translocation. The alternative NF- κ B pathway has been implicated in lymphoid organogenesis and B cell development, and is based in the processing of p100 NF- κ B by IKK α , resulting in release of the p52 NF- κ B bound to RelB [71].

Andrographolide reduces the luciferase activity controlled by NF- κ B and inhibits the DNA binding of NF- κ B induced by chemoattractants, however not affecting I κ B degradation [21]. The detailed mechanism of DNA binding inhibition indicates that andrographolide form a covalent adduct with reduced cysteine 62 of p50 subunit NF- κ B, which block the binding of NF- κ B to DNA [72]. The NF- κ B pathway inhibition by andrographolide has been described in different cells involving in inflammatory processes such as endothelial cells [62], monocytes [73], bronchial epithelial cells [20], and dendritic cells [58].

A second mechanism describes an inhibitory effect of andrographolide on iNOS and COX-2 expression in macrophages, attributable to the modulation of transcription factors AP-1 and STAT3. AP-1 and STAT3, which are important for the production of pro-inflammatory cytokines such as IL-1 β , IL-6 and IL-10, plays a major role in Rheumatoid Arthritis. It has been reported an overexpression of activated STAT3 and high DNA binding activity of AP-1 in synovial tissue from patients with Rheumatoid Arthritis [74, 75]. In fact, andrographolide reduced the LPS-induced AP-1 DNA-binding activities, and also decreased the STAT3 phosphorylation, which is crucial for nuclear translocation and DNA binding [56]. Thus, andrographolide may also be contributing to reduce the inflammatory process in rheumatoid arthritis via AP-1 and/or STAT3 modulation.

A third mechanism involves the interference of the transcription factor Nuclear Factor of Activated T cells (NFAT) induced by andrographolide in T-cells. The interference of NFAT activation by andrographolide is related to the increase of andrographolide-induced JNK phosphorylation, which controls the export of NFAT from nucleus [57].

In addition to the immunomodulatory andrographolide mechanism described above, there are several cellular pathways, such as PI3K/Akt and ERK1/2 pathways, involved in the anti-inflammatory effect of andrographolide and in the pathogenesis of the Rheumatoid Arthritis

[76]. The PI3 kinase pathway, is activated by TNF- α and IL-1, within fibroblastic synovial cells, and can activate the transcription factors NF- κ B and AP-1 [77]. Also, the participation of the ERK1/2 MAPK in the initiation and progression of rheumatoid arthritis suggest that ERK inhibitors may emerge as a new therapeutic tool. The use of an ERK inhibitor in the animal model of collagen-induced arthritis suppressed the antigen-specific activation of T cells [78]. *In vitro*, andrographolide reduced the Akt phosphorylation in macrophages, HUVEC and microglia, and decreased the ERK1/2 phosphorylation in macrophages, suggesting that the signaling pathways PI3K/Akt and ERK1/2 may be associated to its anti-inflammatory effect [24, 61, 79]. Additionally, andrographolide also have the ability to reduce ERK1 and ERK5 phosphorylation [57].

In the following figure we propose the main anti-inflammatory effects of andrographolide that include the inhibition of several intracellular signaling pathways (Figure 3).

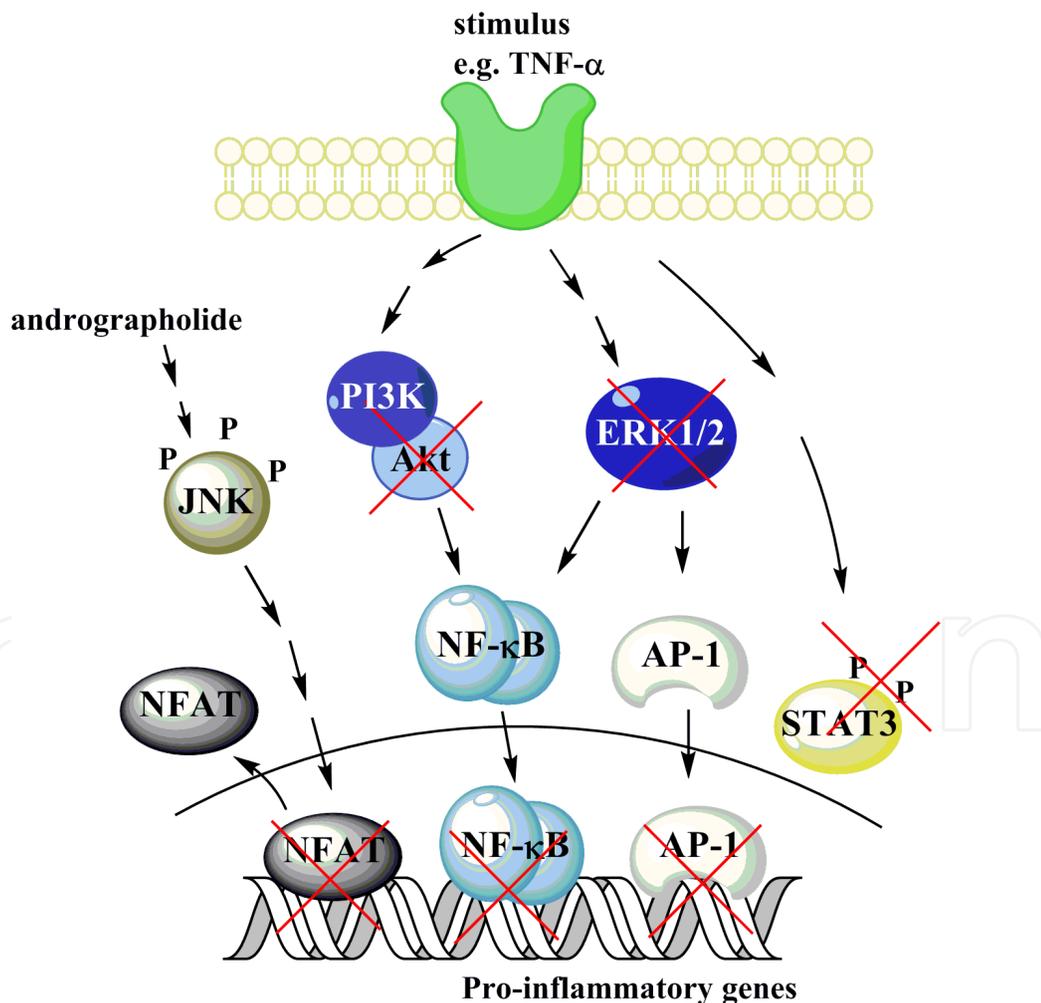


Figure 3. Proposed molecular mechanism of andrographolide in inflammation. Andrographolide shows inhibitory effect (x) on the PI3K/Akt pathway, ERK1/2 MAPK, NF- κ B, NFAT, AP-1 and STAT3, and increases the JNK phosphorylation.

5. Effect of andrographolide on rheumatoid arthritis

5.1. Efficacy of an *Andrographis paniculata* composition (Paractin®) for the relief of rheumatoid arthritis symptoms: A prospective randomized placebo-controlled trial

In a prospective, double blind against placebo controlled clinical trial with chronic active Rheumatoid arthritis, the effect of a standardized patented *A. paniculata* extract (Paractin®) administration to 60 patients during 14 weeks in the reduction of symptoms and signs was studied. Each patient received either a tablet containing 30 mg of andrographolide or a placebo 3 times a day. The demographic characteristic of the patients is shown in table 2.

	Treatment groups	
	Placebo	Active drug
Number of patients	28	30
Age (mean years) (min-max)	44.82 (13-63)	47.1 (20-70)
Years with diagnosed (min-max)	6.5 (0.7-22.3)	6.7 (0.7-44.5)
BMI (Kg/m ²) (min-max)	30.0 (19.7-41.4)	29.2 (18.3-44.5)
Height (m) (min-max)	1.52 (1.30-1.75)	1.51 (1.38-1.69)
Weight (kg) (min-max)	69.9 (43.0-106.0)	67.2 (39.5-100.0)
Intake of NSAIDs, n (%)	17 (60.7%)	18 (60.0%)

Table 1. Demographic characteristics of Rheumatoid Arthritis patients included in the double blind study of *A. paniculata* standardized extract (modified from Burgos et al., 2009).

The results of the study show a significant reduction at the end of the treatment in tender joint, number of swollen joints, total grade of swollen joint, number of tender joints, total grade of swollen joints, total grade of tender joints HAQ 0.52 and SF36 (two health questionnaires) within the group treated with the active drug when comparing day 0 against week 14 (figure 4). The effect was associated to a reduction of rheumatoid factor, IgA, and C4. The study concludes that the drug was significantly effective in reducing symptoms and serological parameters of the disease and therefore useful as natural complement in the treatment of Rheumatoid Arthritis [80].

The clinical efficacy of *A. paniculata* could be explained by the anti-inflammatory properties of andrographolide. Andrographolide present in the extract is a potent inhibitors of NF- κ B [21], a transcription factor linked to pro-inflammatory expression of several proteins such as COX-2, iNOS, and TNF- α , IL-6. Since NF- κ B is involved in the pathogenesis of Rheumatoid Arthritis and other rheumatoid conditions [81], we hypothesized that *A. paniculata* extract tablets (Paractin®) can reduce inflammatory symptoms, signs, serological parameters in these patients. In fact, the clinical findings suggest that the *A. paniculata* formulation may have an additional therapeutical effect over Prednisone and MTX in reducing pain and inflammatory clinical symptoms during treatment period. The beneficial effect in reducing pain and other

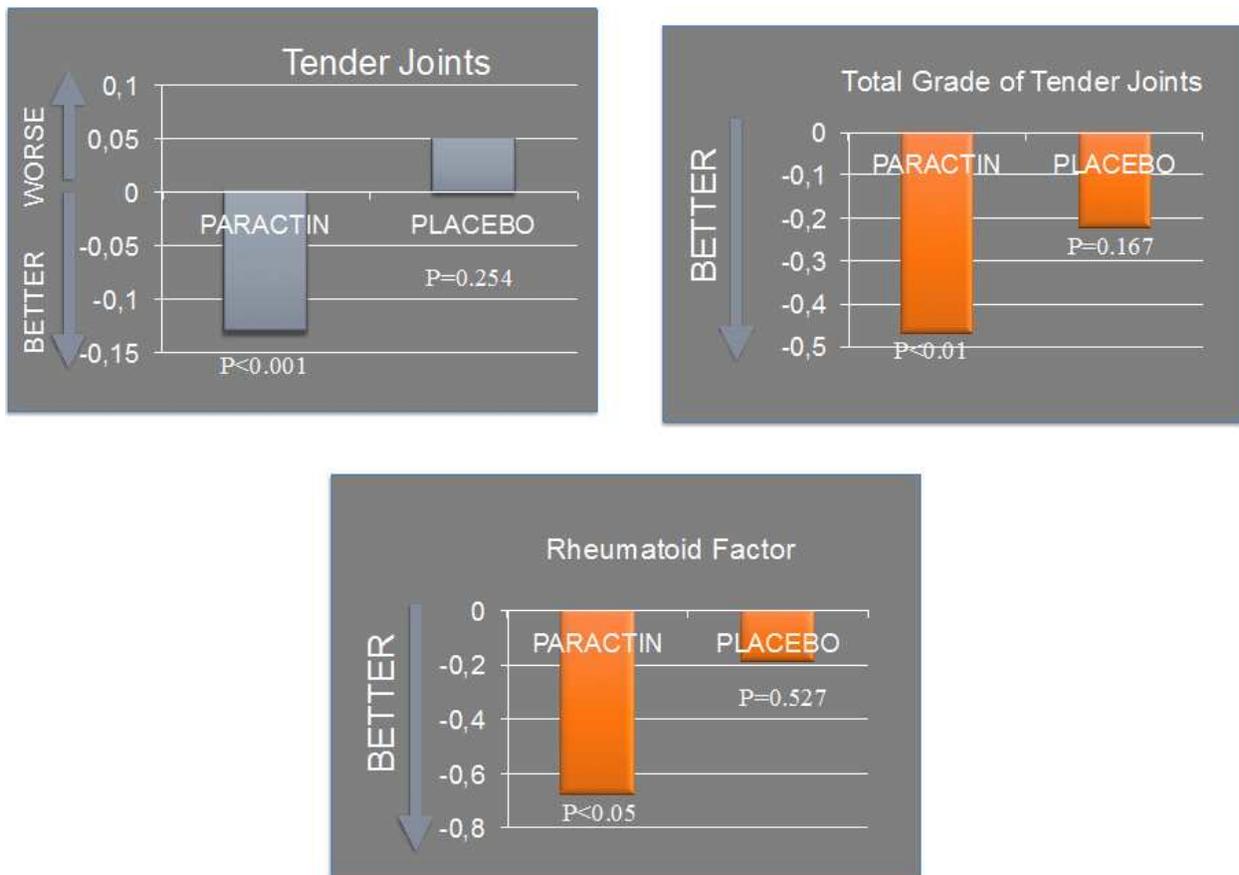


Figure 4. Effect of *A. paniculata* extract (Paractin®) on tender joints, total grade of tender joints and rheumatoid factor [80].

inflammatory symptoms with the *A. paniculata* formulation could be associated to the high standardization of total andrographolides (NLT 30%) in the extract considering. This is closely associated with the inhibition of COX-2 [21] and the reduction of PGE2 production [25], one of the main mechanisms for the control of inflammation and pain in Rheumatoid Arthritis by NSAIDs [82]. The dose of Andrographolide used in the present study was around 1.2 mg per kg. It has been reported that 1mg/kg reaches a steady state plasma concentration of 1.9 μM [83], a concentration able to reduce the PGE2 production [25]. Moreover, in patients treated with *A. paniculata* extract a decrease of rheumatoid factor (RF), creatine kinase, hemoglobin, IgA and IgM were observed. A correlation between RF titers and clinical disease activity has been reported widely [84]. RF titers decrease with methotrexate, suggesting an indirect link with disease activity [85]. Andrographolide can reduce the TNF α production in macrophages, an effect that could be associated with the reduction of auto-antibodies. It is known that a reduction of TNF α can diminish significantly the RF levels [86]. The ability of andrographolide to reduce antibody titer has also been demonstrated in other autoimmune diseases such as experimental autoimmune encephalomyelitis and lupus (see above). A reduction of immunoglobulin, such as IgM and IgA, could also be beneficial in long-term treatment because there is a positive correlation between the grade of cartilage damage in active Rheumatoid Arthritis [87] and decrease of RF. Moreover, treatment with DMARDs reduces the level of IgM and IgA

in patients affected with Rheumatoid Arthritis [85]. We propose that *A. paniculata* could be useful in decreasing the radiological progression in long-term treatments of Rheumatoid Arthritis patients. In support of this, andrographolide reduces NFAT activity, a transcription factor linked with bone erosion [88]. In MC3T3, a murine osteoblast cell line, we observed that andrographolide is able to induce differentiation and calcium mineralization, via expression of COX2 (Burgos et al., data unpublished).

On the other hand, no side effects were observed, indicating that *A. paniculata* treatment was safe, non-toxic, and well tolerated. In the literature, side effects associated with *A. paniculata* or andrographolide, administered in higher doses (4-6mg/kg), have caused isolated cases of allergic reactions, tiredness, headache, pruritus/rash, diarrhea, nausea, metallic taste, bitter taste, dry tongue, eyes sensitive to light, decreased short-term memory, dizziness, heartburn, tender lymph nodes, and lymphadenopathy [89]. None of these effects were observed in Rheumatoid Arthritis patients after 14 weeks of treatment [80].

Despite, the fact that was no difference between *A. paniculata* and placebo treatment after 14 weeks, the intragroup analysis showed a significant decrease of clinical symptoms and serological parameters in the *A. paniculata* group. This effect could become more evident in a long term administration of the drug and follow up Rheumatoid Arthritis patients for several years.

5.2. Monotherapy with an *Andrographis paniculata* standardized extract (Paractin®) for the symptomatic relief of different chronic rheumatoid conditions: A prospective case report and long term follow up

5.2.1. Background

Presently, there is no specific or etiological cure for Rheumatoid Arthritis and these other rheumatoid conditions as well, and treatment aims to limit joint damage, prevent loss of function, and decrease pain. Therapies used for these purposes include nonsteroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs (DMARDs), and corticosteroids. The American College of Rheumatology (ACR) Guidelines recommends the administration of DMARD within 3 months of diagnosis and methotrexate (MTX) as the standard treatment in monotherapy or in combination with other DMARDs [90]. MTX, as a standard therapy, induces significant improvement in the number of tender and swollen joints, pain, and functional status, in addition to physician and patient global assessment. The onset of MTX-induced improvement is generally within 3 months in the majority of patients who will eventually respond, and a plateau in the response is often reached after 6 to 12 months. However, as an anti-metabolic agent, MTX may cause adverse events such as cytopenia, serious infections, liver damage and muco-cutaneous problems. The long term use of MTX, is associated with prevalence of significant liver enzymes in aprox. 13% of the patients and 3.7% of the patients discontinue MTX permanently for liver toxicity [91]

Considering that in the clinical study in patients with Rheumatoid arthritis there was a significant decrease in the group with *A. paniculata* in the symptoms over time (after 14 weeks) on the progression of the diseases, it was proposed that long term treatment could

demonstrate a mayor therapeutic response similar to other DMARs treatment. We report six case reports, with different rheumatoid arthritis conditions, that support the fact that *A. paniculata* standardized extract reduces symptoms of chronic joint pain, stiffness and serological inflammatory parameters in a prospective individual case controlled follow up study over a period of 42 months.

5.2.2. Intervention

The drug of botanical origin used for the treatment of these cases is a patented (US patent 8084495) standardized extract of *A. paniculata* known as Paractin®, manufactured and distributed by Herbal Powers (USA). Paractin® contains andrograpolid NLT 30%, neoandrographolide NLT 0.2% and deoxyandrographolide NLT 3%. Paractin® was supplied directly for this study and stored according to the instructions of the manufacturer. The batch number for the *A. paniculata* extract used in this study was PAR-070801-2. A secondary and identical batch was retained (N° 20050520) and kept at Herbal Powers. Each tablet contained 150 mg of the extract. During all duration of this treatment, two tablets were given before meals three times a day. This dosage regimen was determined in previous preclinical and clinical trials with the pure compound and other commercially available *A. paniculata* extracts [80, 83]. The content of these compounds was evaluated by HPLC using reference standards as described elsewhere [92].

5.2.3. Patients and method

The group consisted of 6 (five adults and one pediatric) patients, 3 male and 3 female, all with a long history of active diseases as shown in Table 2.

Patient	Sex	Age at Diagnosis Year. "0"	Diagnosis	Prevalence of Disease (Years)	Duration of Treatment (Months)
1	Female	51	Rheumatoid Arthritis	6	50
2	Male	36	Rheumatoid Spondylitis	7	50
3	Female	15	Rheumatoid Arthritis/Vasculitis	3	48
4	Female	39	Psoriatic Arthritis	15	60
5	Male	67	Rheumatoid Arthritis/ Serositis	8	38
6	Male	34	Psoriatic Arthritis/ Erythroderma	4	40

Table 2. Antecedents of patients treated with *Andrographis paniculata* standardized extract (Paractin®)

All patients were individually recruited and controlled by their treating physician from the Hospital Regional de Valdivia, Unit of Rheumatology in the city of Valdivia, Chile and complying confirmed diagnosis of Rheumatoid Arthritis conditions before they were enrolled. They all signed a written informed consent, including the one pediatric case that was given

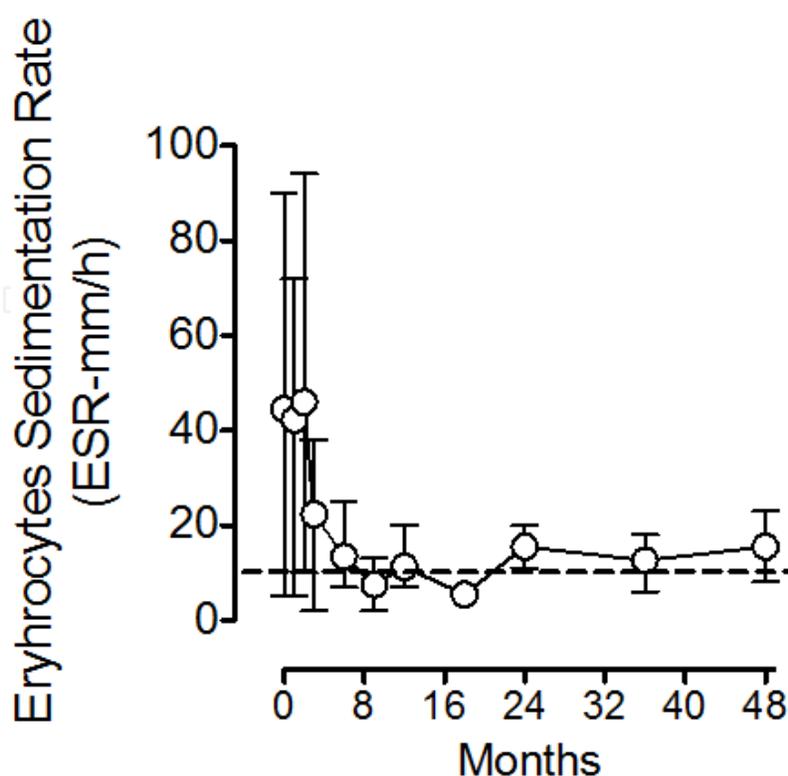


Figure 5. Erythrocyte sedimentation rate (ESR) in patients with chronic Rheumatoid Arthritis compared with ESR value at beginning of treatment. Continuous observation during 48 month. Each point represents the mean and range (maximum-minimum value). In dashed line the normal value.

consent by their parents. Advice and indications to test Paractin® was done by the rheumatologist, who requested the approval of each individual pharmacological protocol and supply of the product. The rationale and main objective was that Paractin® could reduce long term clinical symptoms and serological parameters of inflammation in these patients. Inclusion criteria were confirmed by clinical and laboratory diagnosis, that included active clinical and serological parameters of inflammation, no underlying standard treatment, poor or no response to standard treatment, or important side effects of Methotrexate and Prednisone, like in the female pediatric patient. From day 0, two tablets of Paractin® orally containing 150 mg of standardized *A. paniculata* extract (90 mg andrographolide per day) was administered during 48 month. Total withdrawal of the standard therapy was commonly decided by the treating physician and patient upon improvement observed with Paractin® treatment and informed to the investigators. All patients were controlled monthly during the first six months, then every three months thereafter at their respective place of residence and coordinated by their rheumatologist. After 24 months the treatment with Paractin® tablets, administered orally to patients with Rheumatoid Arthritis, Psoriatic Arthritis and Ankylosing spondylitis, reduced symptoms. In a similar fashion the serum immunological parameters of inflammation were reduced progressively during 48 month of Paractin® treatment.

When Paractin® was given alone; no side effects and good tolerability were observed during the complete period of administration. Only two cases reported a temporary and early and

mild gastric discomfort with the tablets. Plasma biochemical parameters showed normal hematological, liver, kidney and metabolic functions. Interestingly, a moderate reactivation of joint pain and stiffness in two of the Rheumatoid arthritis patients and the one Ankylosing spondylitis patient was observed, due to an interruption of the treatment during 15, 11 and 22 days, respectively. Interestingly, these withdrawal and continuity incidents suggest that after peak and steady efficacy is reached and according to clinical and serological parameters follow up, a residual activity of the product is maintained between two and three weeks, disappearing at week four, and then recovered back again to previous status after four weeks. Also, we have so far not observed any loss of efficacy, or the need to increase dosages of the product, proving that no adaptation or refractoriness has yet been developed in this treated group. After one to five years follow up of these six rheumatologic patients, given a daily monotherapy of three Paractin® – tablets per day, we can conclude this product is well tolerated, safe and efficacious for the symptomatic relief and serological control of underlying inflammation related to their disease activity.

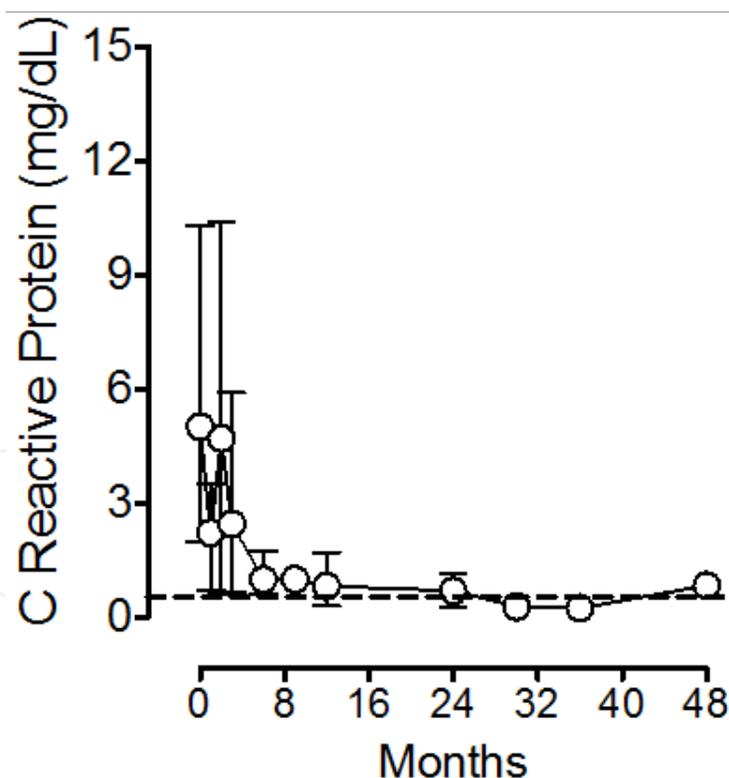


Figure 6. C Reactive protein (CRP) in patients with chronic rheumatoid disease compared with the CRP value at the beginning of treatment with Paractin®. Continuous observation during 48 months. Each point represents the mean and range (maximum-minimum value). In dashed line the normal value.

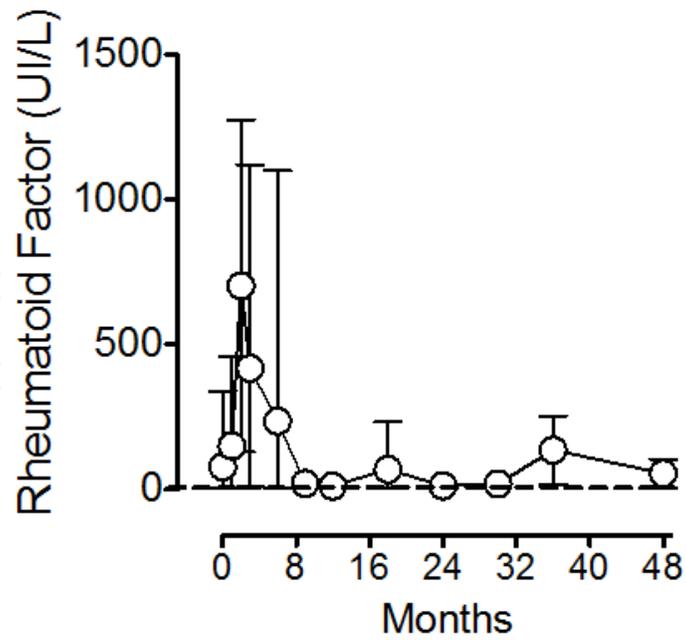


Figure 7. Rheumatoid Factor (RF) in patients with chronic rheumatoid disease treated with Paractin® during 48 month. Each point represents the mean and range (maximum-minimum value). In dashed line the normal value.

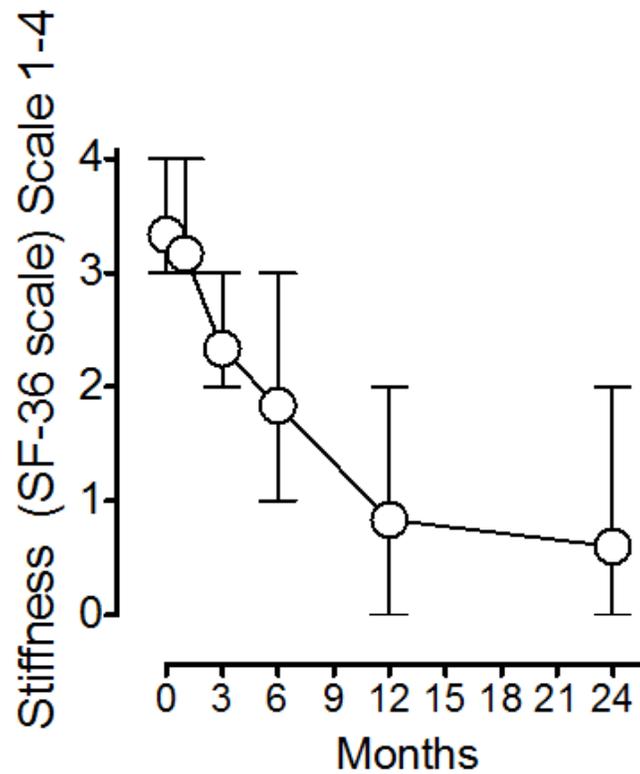


Figure 8. Variation on Rheumatologic stiffness in patients with chronic Rheumatoid Arthritis, treated with Paractin® during 24 month. Each point represents the mean and range (maximum-minimum value).

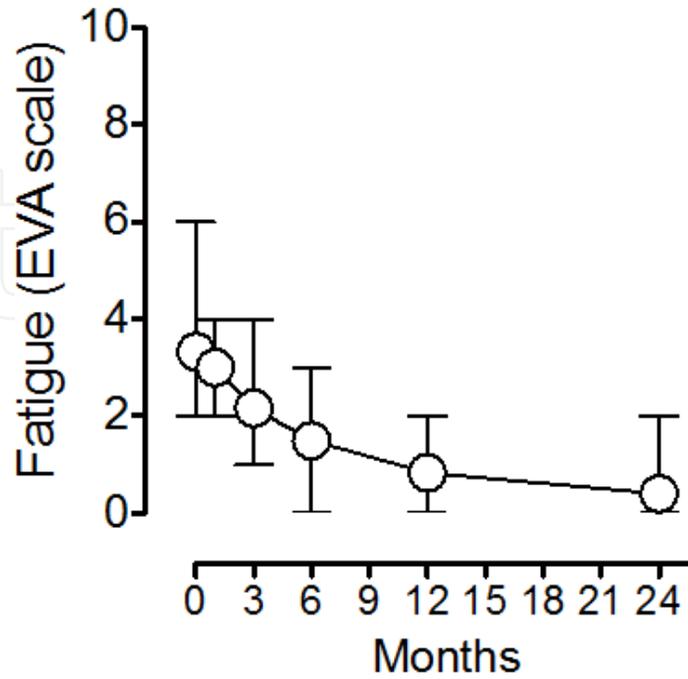


Figure 9. Effect of Paractin® on Fatigue in patients with chronic Rheumatoid Arthritis, treated during 24 month. Each point represents the mean and range (maximum-minimum value).

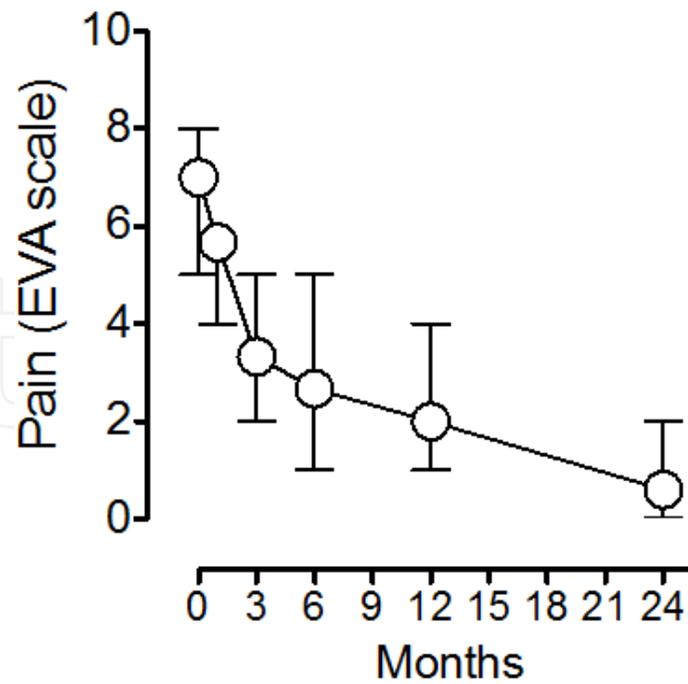


Figure 10. Effect of Paractin® on pain in patients with chronic Rheumatoid Arthritis, treated during 24 month. Each point represents the mean and range (maximum-minimum value).

6. Conclusion

Several studies describe a potent anti-inflammatory action of *Andrographis paniculata* and andrographolide. Andrographolide shows a reduction of the production of pro-inflammatory mediators, such as COX-2, iNOS and cytokines. The molecular mechanism of andrographolide implies the reduction of the activation of transcription factors as NF- κ B, AP-1, STAT3 and NFAT and the inhibition of intracellular signaling pathways. *A. paniculata* standardized extract (30% andrographolide) in clinical trials showed effectiveness for symptom relief and reduce serological parameters in patients with Rheumatoid Arthritis, and the data support a long term treatment similar to other DMARDs.

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References

- [1] Lim, J. C, Chan, T. K, Ng, D. S, Sagineedu, S. R, Stanslas, J, & Wong, W. S. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. *Clin Exp Pharmacol Physiol.* (2012). , 39(3), 300-10.
- [2] Tang, W, & Eisenbrand, G. *Chinese Drugs of Plant Origin.* Berlin: Springer Verlag; (1992).
- [3] Matsuda, T, Kuroyanagi, M, Sugiyama, S, Umehara, K, Ueno, A, & Nishi, K. Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees. *Chem Pharm Bull (Tokyo).* (1994). , 42(6), 1216-25.
- [4] Pramanick, S, Banerjee, S, Achari, B, Das, B, & Sen, A. K. Sr., Mukhopadhyay S, et al. Andropanolide and isoandrographolide, minor diterpenoids from *Andrographis paniculata*: structure and X-ray crystallographic analysis. *J Nat Prod.* (2006). , 69(3), 403-5.

- [5] Sharma, A, Lal, K, & Handa, S. S. Standardization of the indian crude drug kalmegh by high-pressure liquid-chromatographic determination of andrographolide. *Phytochemical Analysis*. (1992). , 3(3), 129-31.
- [6] Reddy, V. L, Reddy, S. M, Ravikanth, V, Krishnaiah, P, Goud, T. V, Rao, T. P, et al. A new bis-andrographolide ether from *Andrographis paniculata* nees and evaluation of anti-HIV activity. *Nat Prod Res*. (2005). , 19(3), 223-30.
- [7] Wiart, C, Kumar, K, Yusof, M. Y, Hamimah, H, Fauzi, Z. M, & Sulaiman, M. Antiviral properties of ent-labdene diterpenes of *Andrographis paniculata* nees, inhibitors of herpes simplex virus type 1. *Phytother Res*. (2005). , 19(12), 1069-70.
- [8] Yang, S, Evens, A. M, Prachand, S, Singh, A. T, Bhalla, S, David, K, et al. Mitochondrial-mediated apoptosis in lymphoma cells by the diterpenoid lactone andrographolide, the active component of *Andrographis paniculata*. *Clin Cancer Res*. (2010). , 16(19), 4755-68.
- [9] Zhou, J, Lu, G. D, Ong, C. S, Ong, C. N, & Shen, H. M. Andrographolide sensitizes cancer cells to TRAIL-induced apoptosis via death receptor 4 up-regulation. *Mol Cancer Ther*. (2008). , 53.
- [10] Yu, B. C, Chang, C. K, Su, C. F, & Cheng, J. T. Mediation of beta-endorphin in andrographolide-induced plasma glucose-lowering action in type I diabetes-like animals. *Naunyn Schmiedebergs Arch Pharmacol*. (2008).
- [11] Zhang, Z, Jiang, J, Yu, P, Zeng, X, Larrick, J. W, & Wang, Y. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. *J Transl Med*. (2009).
- [12] Sheeja, K, Guruvayoorappan, C, & Kuttan, G. Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. *Int Immunopharmacol*. (2007). , 7(2), 211-21.
- [13] Thisoda, P, Rangkadilok, N, Pholphana, N, Worasuttayangkurn, L, Ruchirawat, S, & Satayavivad, J. Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation. *Eur J Pharmacol*. (2006).
- [14] Sheeja, K, & Kuttan, G. Protective effect of *Andrographis paniculata* and andrographolide on cyclophosphamide-induced urothelial toxicity. *Integr Cancer Ther*. (2006). , 5(3), 244-51.
- [15] Sinha, J, Mukhopadhyay, S, Das, N, & Basu, M. K. Targeting of liposomal andrographolide to *L. donovani*-infected macrophages in vivo. *Drug Deliv*. (2000). , 7(4), 209-13.
- [16] Handa, S. S, & Sharma, A. Hepatoprotective activity of andrographolide against galactosamine & paracetamol intoxication in rats. *Indian J Med Res*. (1990). , 92, 284-92.
- [17] Handa, S. S, & Sharma, A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbontetrachloride. *Indian J Med Res*. (1990). , 92, 276-83.

- [18] Singha, P. K, Roy, S, & Dey, S. Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice. *J Ethnopharmacol.* (2007). , 111(1), 13-21.
- [19] Woo, A. Y, Waye, M. M, Tsui, S. K, Yeung, S. T, & Cheng, C. H. Andrographolide up-regulates cellular-reduced glutathione level and protects cardiomyocytes against hypoxia/reoxygenation injury. *J Pharmacol Exp Ther.* (2008). , 325(1), 226-35.
- [20] Bao, Z, Guan, S, Cheng, C, Wu, S, Wong, S. H, Kemeny, D. M, et al. A novel anti-inflammatory role for andrographolide in asthma via inhibition of the nuclear factor-kappaB pathway. *Am J Respir Crit Care Med.* (2009). , 179(8), 657-65.
- [21] Hidalgo, M. A, Romero, A, Figueroa, J, Cortes, P, Concha, I. I, Hancke, J. L, et al. Andrographolide interferes with binding of nuclear factor-kappaB to DNA in HL-60-derived neutrophilic cells. *Br J Pharmacol.* (2005). , 144(5), 680-6.
- [22] Li, J, Luo, L, Wang, X, Liao, B, & Li, G. Inhibition of NF-kappaB expression and allergen-induced airway inflammation in a mouse allergic asthma model by andrographolide. *Cell Mol Immunol.* (2009). , 6(5), 381-5.
- [23] Parichatikanond, W, Suthisisang, C, Dhepakson, P, & Herunsalee, A. Study of anti-inflammatory activities of the pure compounds from *Andrographis paniculata* (burm.f.) Nees and their effects on gene expression. *Int Immunopharmacol.* (2010). , 10(11), 1361-73.
- [24] Qin, L. H, Kong, L, Shi, G. J, Wang, Z. T, & Ge, B. X. Andrographolide inhibits the production of TNF-alpha and interleukin-12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinases. *Biol Pharm Bull.* (2006). , 29(2), 220-4.
- [25] Wang, T, Liu, B, Zhang, W, Wilson, B, & Hong, J. S. Andrographolide reduces inflammation-mediated dopaminergic neurodegeneration in mesencephalic neuron-glia cultures by inhibiting microglial activation. *J Pharmacol Exp Ther.* (2004). , 308(3), 975-83.
- [26] Batkhuu, J, Hattori, K, Takano, F, Fushiya, S, Oshiman, K, & Fujimiya, Y. Suppression of NO production in activated macrophages in vitro and ex vivo by neoandrographolide isolated from *Andrographis paniculata*. *Biol Pharm Bull.* (2002). , 25(9), 1169-74.
- [27] Liu, J, Wang, Z. T, & Ji, L. L. In vivo and in vitro anti-inflammatory activities of neoandrographolide. *Am J Chin Med.* (2007). , 35(2), 317-28.
- [28] Pfisterer, P. H, Rollinger, J. M, Schyschka, L, Rudy, A, Vollmar, A. M, & Stuppner, H. Neoandrographolide from *Andrographis paniculata* as a potential natural chemosensitizer. *Planta Med.* (2010). , 76(15), 1698-700.
- [29] Kamdem, R. E, Sang, S, & Ho, C. T. Mechanism of the superoxide scavenging activity of neoandrographolide- a natural product from *Andrographis paniculata* Nees. *J Agric Food Chem.* (2002). , 50(16), 4662-5.

- [30] Yoopan, N, Thisoda, P, Rangkadilok, N, Sahasitiwat, S, Pholphana, N, Ruchirawat, S, et al. Cardiovascular effects of 14-deoxy-11,12-didehydroandrographolide and *Andrographis paniculata* extracts. *Planta Med.* (2007). , 73(6), 503-11.
- [31] Zhang, C, Kuroyangi, M, & Tan, B. K. Cardiovascular activity of 14-deoxy-11,12-didehydroandrographolide in the anaesthetised rat and isolated right atria. *Pharmacol Res.* (1998). , 38(6), 413-7.
- [32] Akowuah, G. A, Zhari, I, Mariam, A, & Yam, M. F. Absorption of andrographolides from *Andrographis paniculata* and its effect on CCl₄-induced oxidative stress in rats. *Food Chem Toxicol.* (2009). , 47(9), 2321-6.
- [33] Thamlikitkul, V, Dechatiwongse, T, Theerapong, S, Chantrakul, C, Boonroj, P, Punkrut, W, et al. Efficacy of *Andrographis paniculata*, Nees for pharyngotonsillitis in adults. *J Med Assoc Thai.* (1991). , 74(10), 437-42.
- [34] Lee, M. J, Rao, Y. K, Chen, K, Lee, Y. C, Chung, Y. S, & Tzeng, Y. M. Andrographolide and 14-deoxy-11,12-didehydroandrographolide from *Andrographis paniculata* attenuate high glucose-induced fibrosis and apoptosis in murine renal mesangial cell lines. *J Ethnopharmacol.* (2010). , 132(2), 497-505.
- [35] Roy, D. N, Mandal, S, Sen, G, Mukhopadhyay, S, & Biswas, T. Deoxyandrographolide desensitizes hepatocytes to tumour necrosis factor-alpha-induced apoptosis through calcium-dependent tumour necrosis factor receptor superfamily member 1A release via the NO/cGMP pathway. *Br J Pharmacol.* (2010). , 160(7), 1823-43.
- [36] Burgos, R. A, Loyola, M, Hidalgo, M. A, Labranche, T. P, & Hancke, J. L. Effect of 14-deoxyandrographolide on calcium-mediated rat uterine smooth muscle contractility. *Phytother Res.* (2003). , 17(9), 1011-5.
- [37] Naik, S. R, & Hule, A. Evaluation of immunomodulatory activity of an extract of andrographolides from *Andrographis paniculata*. *Planta Med.* (2009). , 75(8), 785-91.
- [38] Burgos, R. A, Hidalgo, M. A, & Monsalve, J. LaBranche TP, Eyre P, Hancke JL. 14-deoxyandrographolide as a platelet activating factor antagonist in bovine neutrophils. *Planta Med.* (2005). , 71(7), 604-8.
- [39] Sriramaneni, R. N, Omar, A. Z, Ibrahim, S. M, & Amirin, S. Mohd Zaini A. Vasorelaxant effect of diterpenoid lactones from *Andrographis paniculata* chloroform extract on rat aortic rings. *Pharmacognosy Res.* (2010). , 2(4), 242-6.
- [40] Lala, S, Nandy, A. K, Mahato, S. B, & Basu, M. K. Delivery in vivo of 14-deoxy-11-oxoandrographolide, an antileishmanial agent, by different drug carriers. *Indian J Biochem Biophys.* (2003). , 40(3), 169-74.
- [41] Kapil, A, Koul, I. B, Banerjee, S. K, & Gupta, B. D. Antihepatotoxic effects of major diterpenoid constituents of *Andrographis paniculata*. *Biochem Pharmacol.* (1993). , 46(1), 182-5.
- [42] Ji, L. L, Wang, Z, Dong, F, Zhang, W. B, & Wang, Z. T. Andrograpanin, a compound isolated from anti-inflammatory traditional Chinese medicine *Andrographis panicu-*

- lata, enhances chemokine SDF-1 α -induced leukocytes chemotaxis. *J Cell Biochem.* (2005). , 95(5), 970-8.
- [43] Liu, J, Wang, Z. T, & Ge, B. X. Andrograpanin, isolated from *Andrographis paniculata*, exhibits anti-inflammatory property in lipopolysaccharide-induced macrophage cells through down-regulating the MAPKs signaling pathways. *Int Immunopharmacol.* (2008). , 38.
- [44] He, X. J, Zeng, X. B, Hu, H, & Wu, Y. X. Cytotoxic biotransformed products from andrographolide by *Rhizopus stolonifer* ATCC 12939. *Journal of Molecular Catalysis B-Enzymatic.* (2010).
- [45] Li, W, Xu, X, Zhang, H, Ma, C, Fong, H, Van Breemen, R, et al. Secondary metabolites from *Andrographis paniculata*. *Chem Pharm Bull (Tokyo).* (2007). , 55(3), 455-8.
- [46] Suebsasana, S, Pongnaratorn, P, Sattayasai, J, Arkaravichien, T, Tiamkao, S, & Aromdee, C. Analgesic, antipyretic, anti-inflammatory and toxic effects of andrographolide derivatives in experimental animals. *Arch Pharm Res.* (2009). , 32(9), 1191-200.
- [47] Xu, J, Huang, S, Luo, H, Li, G, Bao, J, Cai, S, et al. QSAR Studies on andrographolide derivatives as α -glucosidase inhibitors. *Int J Mol Sci.* (2010). , 11(3), 880-95.
- [48] Zhou, B, Zhang, D, & Wu, X. Biological Activities and Corresponding Sar Analysis of Andrographolide and its Derivatives. *Mini Rev Med Chem.* (2012).
- [49] Kanniappan, M, Mathuram, L. N, & Natarajan, R. A study on the antipyretic effect of chiretta (*andrographis-paniculata*). *Indian Veterinary Journal.* (1991). , 68(4), 314-6.
- [50] Shen, Y. C, Chen, C. F, & Chiou, W. F. Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide. *Planta Med.* (2000). , 66(4), 314-7.
- [51] Shen, Y. C, Chen, C. F, & Chiou, W. F. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. *Br J Pharmacol.* (2002). , 135(2), 399-406.
- [52] Abu-ghefreh, A. A, Canatan, H, & Ezeamuzie, C. I. In vitro and in vivo anti-inflammatory effects of andrographolide. *Int Immunopharmacol.* (2009). , 9(3), 313-8.
- [53] Tsai, H. R, Yang, L. M, Tsai, W. J, & Chiou, W. F. Andrographolide acts through inhibition of ERK1/2 and Akt phosphorylation to suppress chemotactic migration. *Eur J Pharmacol.* (2004).
- [54] Nagy, G, Koncz, A, Talarico, T, Fernandez, D, Ersek, B, Buzas, E, et al. Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Res Ther.* (2010).
- [55] Chiou, W. F, Lin, J. J, & Chen, C. F. Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with lipopolysaccharide. *Br J Pharmacol.* (1998). , 125(2), 327-34.

- [56] Lee, K. C, Chang, H. H, Chung, Y. H, & Lee, T. Y. Andrographolide acts as an anti-inflammatory agent in LPS-stimulated RAW264.7 macrophages by inhibiting STAT3-mediated suppression of the NF-kappaB pathway. *J Ethnopharmacol.* (2011). , 135(3), 678-84.
- [57] Carretta, M. D, Alarcon, P, Jara, E, Solis, L, Hancke, J. L, Concha, I. I, et al. Andrographolide reduces IL-2 production in T-cells by interfering with NFAT and MAPK activation. *Eur J Pharmacol.* (2009).
- [58] Iruretagoyena, M. I, Sepulveda, S. E, Lezana, J. P, Hermoso, M, Bronfman, M, Gutierrez, M. A, et al. Inhibition of nuclear factor-kappa B enhances the capacity of immature dendritic cells to induce antigen-specific tolerance in experimental autoimmune encephalomyelitis. *J Pharmacol Exp Ther.* (2006). , 318(1), 59-67.
- [59] Iruretagoyena, M. I, Tobar, J. A, Gonzalez, P. A, Sepulveda, S. E, Figueroa, C. A, Burgos, R. A, et al. Andrographolide interferes with T cell activation and reduces experimental autoimmune encephalomyelitis in the mouse. *J Pharmacol Exp Ther.* (2005). , 312(1), 366-72.
- [60] Burgos, R. A, Seguel, K, Perez, M, Meneses, A, Ortega, M, Guarda, M. I, et al. Andrographolide inhibits IFN-gamma and IL-2 cytokine production and protects against cell apoptosis. *Planta Med.* (2005). , 71(5), 429-34.
- [61] Chen, H. W, Lin, A. H, Chu, H. C, Li, C. C, Tsai, C. W, Chao, C. Y, et al. Inhibition of TNF-alpha-Induced Inflammation by andrographolide via down-regulation of the PI3K/Akt signaling pathway. *J Nat Prod.* (2011). , 74(11), 2408-13.
- [62] Chao, C. Y, Lii, C. K, Tsai, I. T, Li, C. C, Liu, K. L, Tsai, C. W, et al. Andrographolide inhibits ICAM-1 expression and NF-kappaB activation in TNF-alpha-treated EA.hy926 cells. *J Agric Food Chem.* (2011). , 59(10), 5263-71.
- [63] Pratheeshkumar, P, & Kuttan, G. Andrographolide inhibits human umbilical vein endothelial cell invasion and migration by regulating MMP-2 and MMP-9 during angiogenesis. *J Environ Pathol Toxicol Oncol.* (2011). , 30(1), 33-41.
- [64] Chen, J. H, Hsiao, G, Lee, A. R, Wu, C. C, & Yen, M. H. Andrographolide suppresses endothelial cell apoptosis via activation of phosphatidyl inositol-3-kinase/Akt pathway. *Biochem Pharmacol.* (2004). , 67(7), 1337-45.
- [65] Yan, J, Chen, Y, He, C, Yang, Z. Z, Lu, C, & Chen, X. S. Andrographolide induces cell cycle arrest and apoptosis in human rheumatoid arthritis fibroblast-like synoviocytes. *Cell Biol Toxicol.* (2012). , 28(1), 47-56.
- [66] Burgos, R. A, Hidalgo, M. A, Carretta, M. D, Bertoglio, J. C, Folch, H, & Hancke, J. L. Immunomodulatory activities induced by *Andrographis paniculata*. Govil JN, Singh VK, editors. USA: Studium Press LLC; (2009).
- [67] Carreno, L. J, Riedel, C. A, & Kalergis, A. M. Induction of tolerogenic dendritic cells by NF-kappaB blockade and Fcgamma receptor modulation. *Methods Mol Biol.* (2011). , 677, 339-53.

- [68] Kalergis, A. M, Iruretagoyena, M. I, Barrientos, M. J, Gonzalez, P. A, Herrada, A. A, Leiva, E. D, et al. Modulation of nuclear factor-kappaB activity can influence the susceptibility to systemic lupus erythematosus. *Immunology*. (2009). Suppl):e, 306-14.
- [69] Szekanecz, Z, & Koch, A. E. Mechanisms of Disease: angiogenesis in inflammatory diseases. *Nat Clin Pract Rheumatol*. (2007). , 3(11), 635-43.
- [70] Kumar, S, Patil, H. S, Sharma, P, Kumar, D, Dasari, S, Puranik, V. G, et al. Andrographolide Inhibits Osteopontin Expression and Breast Tumor Growth Through Down Regulation of PI3 kinase/Akt Signaling Pathway. *Curr Mol Med*. (2012).
- [71] Vallabhapurapu, S, & Karin, M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol*. (2009). , 27, 693-733.
- [72] Xia, Y. F, Ye, B. Q, Li, Y. D, Wang, J. G, He, X. J, Lin, X, et al. Andrographolide attenuates inflammation by inhibition of NF-kappa B activation through covalent modification of reduced cysteine 62 of J Immunol. (2004). , 50.
- [73] Lee, W. R, Chung, C. L, Hsiao, C. J, Chou, Y. C, Hsueh, P. J, Yang, P. C, et al. Suppression of matrix metalloproteinase-9 expression by andrographolide in human monocytic THP-1 cells via inhibition of NF-kappaB activation. *Phytomedicine*. (2012).
- [74] Shouda, T, Yoshida, T, Hanada, T, Wakioka, T, Oishi, M, Miyoshi, K, et al. Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. *J Clin Invest*. (2001). , 108(12), 1781-8.
- [75] Asahara, H, Fujisawa, K, Kobata, T, Hasunuma, T, Maeda, T, Asanuma, M, et al. Direct evidence of high DNA binding activity of transcription factor AP-1 in rheumatoid arthritis synovium. *Arthritis Rheum*. (1997). , 40(5), 912-8.
- [76] Tas, S. W, Remans, P. H, Reedquist, K. A, & Tak, P. P. Signal transduction pathways and transcription factors as therapeutic targets in inflammatory disease: towards innovative antirheumatic therapy. *Curr Pharm Des*. (2005). , 11(5), 581-611.
- [77] Morel, J, & Berenbaum, F. Signal transduction pathways: new targets for treating rheumatoid arthritis. *Joint Bone Spine*. (2004). , 71(6), 503-10.
- [78] Otori, M. ERK inhibitors as a potential new therapy for rheumatoid arthritis. *Drug News Perspect*. (2008). , 21(5), 245-50.
- [79] Chern, C. M, Liou, K. T, Wang, Y. H, Liao, J. F, Yen, J. C, & Shen, Y. C. Andrographolide inhibits PI3K/AKT-dependent NOX2 and iNOS expression protecting mice against hypoxia/ischemia-induced oxidative brain injury. *Planta Med*. (2011). , 77(15), 1669-79.
- [80] Burgos, R. A, Hancke, J. L, Bertoglio, J. C, Aguirre, V, Arriagada, S, Calvo, M, et al. Efficacy of an *Andrographis paniculata* composition for the relief of rheumatoid arthritis symptoms: a prospective randomized placebo-controlled trial. *Clin Rheumatol*. (2009). , 28(8), 931-46.
- [81] Handel, M. L, Mcmorrow, L. B, & Gravalles, E. M. Nuclear factor-kappa B in rheumatoid synovium. Localization of and p65. *Arthritis Rheum*. (1995). , 50.

- [82] Daoud, K. F, Jackson, C. G, & Williams, H. J. Basic therapy for rheumatoid arthritis: nonsteroidal anti-inflammatory drugs. *Compr Ther.* (1999).
- [83] Panossian, A, Hovhannisyan, A, Mamikonyan, G, Abrahamian, H, Hambardzumyan, E, Gabrielian, E, et al. Pharmacokinetic and oral bioavailability of andrographolide from *Andrographis paniculata* fixed combination Kan Jang in rats and human. *Phytomedicine.* (2000). , 7(5), 351-64.
- [84] Vittecoq, O, Pouplin, S, Krzanowska, K, Jouen-beades, F, Menard, J. F, Gayet, A, et al. Rheumatoid factor is the strongest predictor of radiological progression of rheumatoid arthritis in a three-year prospective study in community-recruited patients. *Rheumatology (Oxford).* (2003). , 42(8), 939-46.
- [85] Olsen, N. J, Teal, G. P, & Brooks, R. H. IgM-rheumatoid factor and responses to second-line drugs in rheumatoid arthritis. *Agents Actions.* (1991).
- [86] Yazdani-biuki, B, Stadlmaier, E, Mulabecirovic, A, Brezinschek, R, Tilz, G, Demel, U, et al. Blockade of tumour necrosis factor {alpha} significantly alters the serum level of IgG- and IgA-rheumatoid factor in patients with rheumatoid arthritis. *Ann Rheum Dis.* (2005). , 64(8), 1224-6.
- [87] He, Y, Zha, Q, Liu, D, & Lu, A. Relations between serum IgA level and cartilage erosion in 436 cases of rheumatoid arthritis. *Immunol Invest.* (2007). , 36(3), 285-91.
- [88] Pessler, F, Dai, L, Cron, R. Q, & Schumacher, H. R. NFAT transcription factors--new players in the pathogenesis of inflammatory arthropathies? *Autoimmun Rev.* (2006). , 5(2), 106-10.
- [89] Coon, J. T, & Ernst, E. *Andrographis paniculata* in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy. *Planta Med.* (2004). , 70(4), 293-8.
- [90] Guidelines for the management of rheumatoid arthritis: (2002). *UpdateArthritis Rheum.* 2002;; 46(2), 328-46.
- [91] Salliot, C, & Van Der Heijde, D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. *Ann Rheum Dis.* (2009). , 68(7), 1100-4.
- [92] Burgos, R. A, Caballero, E. E, Sanchez, N. S, Schroeder, R. A, Wikman, G. K, & Hancke, J. L. Testicular toxicity assessment of *Andrographis paniculata* dried extract in rats. *J Ethnopharmacol.* (1997). , 58(3), 219-24.