Clin Rheumatol
DOI 10.1007/s10067-009-1180-5

ORIGINAL ARTICLE

Efficacy of an *Andrographis paniculata* composition for the relief of rheumatoid arthritis symptoms: a prospective randomized placebo-controlled trial

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Received: 2 December 2008 / Revised: 13 February 2009 / Accepted: 27 March 2009

Abstract *Andrographis paniculata* (Burm. f.) Wall ex Nees (Acanthaceae) possesses anti-inflammatory effects, attributed to the main constituent andrographolide proposed as alternative in the treatment of autoimmune disease. A prospective, randomized, double blind, and placebo-controlled study in patients with rheumatoid arthritis (RA) was performed. Tablets (Paractin®) made of an extract of *A. paniculata* (30% total andrographolides) were administered three times a day for 14 weeks, after a 2-week washout period to 60 patients with active RA. The primary outcomes were pain intensity measured using a horizontal visual analog pain scale (VAPS). In addition, ACR, EULAR, and SF36 clinical parameters were recorded. The intensity of joint pain decreased in the active vs placebo group at the end of treatment, although these differences were not statistically significant. A significant diminishing for week in tender joint −0.13 95% confidence interval (CI; −0.22 to 0.06; p=0.001), number of swollen joints −0.15 95%CI (−0.29 to −0.02; p=0.02), total grade of swollen joint −0.27 95%CI (−0.48 to −0.07; p=0.010), number of tender joints −0.25 95%CI (−0.48 to −0.02; p=0.033), total grade of swollen joints −0.27 95%CI (−0.48 to −0.07; p=0.01), total grade of tender joints −0.47 95%CI (−0.77 to −0.17; p=0.002) and HAQ −0.52 95%CI (−0.82 to −0.21; p<0.001) and SF36 0.02 95% CI (0.01 to 0.02; p<0.001) health questionnaires was observed within the group with the active drug. Moreover, it was associated to a reduction of rheumatoid factor, IgA, and C4. These findings suggest that *A. paniculata* could be a useful "natural complement" in the treatment of AR; however, a larger trial and a more extended period of treatment is necessary in order to corroborate these results.

Keywords Andrographis · Rheumatoid arthritis

Registration trial no. NCT00749645 (ClinicalTrials. Gov)

This work was financed by CONICYT/FONDEF D04I1240 grant and INNOVA CHILE CORFO 06FC01IPC-91, Patagonia Clinical Trial-CRO, Valdivia, Chile.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory systemic autoimmune disease that has a global distribution

Published online: 29 April 2009

with an estimated prevalence of 0.5% to 2%, being two to three times greater in women than in men [1, 2]. In Chile, the prevalence is about 2% [3]. This is characterized by symmetric arthritis of arthrodial joints, leading to progressive erosion of cartilage and bone [4]. There is no cure for RA, and treatment aims are limiting joint damage, preventing loss of function, and decreasing pain. Drugs used for these purposes include nonsteroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs (DMARDs), and corticosteroids. Unfortunately, joint destruction can often progress despite treatment, leading to deformity and disability in a substantial number of patients. In recent years, several studies have shown that a greater impact on slowing disease progression can be achieved if patients with recent-onset RA are treated with DMARDs earlier than it had previously been recommended [5]. Hence, the current American College of Rheumatology (ACR) guidelines recommend initiation of DMARD treatment within 3 months of diagnosis and methotrexate (MTX) as the standard in monotherapy or in combination with other DMARDs [6]. 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 [7].

Andrographis paniculata Nees, a shrub belonging to the Acanthaceae family has been widely used in the Ayurvedic and Chinese systems of medicine for thousands of years as treatment for malaria, diarrhea, hepatitis, multiple infections, and digestive disorders [8, 9]. Andrographolide, the main labdane diterpene found in the aerial part of the plant, is thought to be responsible for the different biological effects. Recently, dried standardized extracts containing total andrographolides or pure andrographolide have been used for the treatment of viral infections and inflammatory diseases [8]. In this sense, andrographolide is known to exert several anti-inflammatory properties, including inhibition of intercellular adhesion molecule-1 expression in monocytes activated by tumor necrosis factor-α [10], suppression of inducible nitric oxide synthetase (iNOS) in RAW264,7 [11], COX2 expression in neutrophils and microglial cells [12, 13], and tumor necrosis factor alpha (TNF-α) [14], interferon gamma (IFN-γ), and interleukin-2 (IL-2) production [15, 16]. In addition, andrographolide can interfere with proinflammatory gene expression, affecting signal transduction pathways such as MAPK/ERK1/2 [17, 18] or PI3K/Akt in macrophages [17]. Furthermore, andrographolide reduces ERK1/2 phosphorylation in murine T cells [15], an intracellular signaling pathway involved in the cytokine expression, i.e., IL-2, TNF-α, and IFN-γ [19, 20].

It has been proposed that andrographolide exerts its anti-inflammatory effects by inhibiting nuclear factor kappa B (NF-κB) binding to DNA and thus reducing the expression of proinflammatory proteins in neutrophils [12]. Recently, we have demonstrated that andrographolide reduced IL-2 production in Jurkat cells stimulated with phorbol 12-myristate 13- acetate /ionomycin. In addition, andrographolide reduced nuclear factor of activated T cell (NFAT) luciferase activity and interfered with its nuclear distribution, all these effects being linked to an increase in JNK phosphorylation [21].

In RA, the transcription factors NF-κB and NFAT have been recognized as important factors in regulating the inflammatory processes and progression of the disease [22, 23]. In vivo studies suggest that IKKβ inhibition, a factor that regulates the activity of NF-κB, is an effective therapeutic approach to treat both inflammation and bone/cartilage destruction observed in a rat model of RA [24]. On the other hand, the most convincing evidence that NFATs may be important in the pathogenesis or perpetuation of inflammatory arthropathies stems from the observation that treatment with Ciclosporin A is effective in otherwise refractory RA [25, 26].

In the present clinical trial, we assess the clinical effectiveness of an extract of *A. paniculata* tablets standardized to 30% andrographolides in patients affected with RA during 3 months.

Materials and methods

Participants

Patients were recruited from two rheumatology hospital units in the cities of Valdivia and Osorno. Selected patients presented RA and fulfilled the ACR 1987 criteria for diagnosis of RA [27]. They were enrolled between October 7th, 2006 and August 30th, 2007. Inclusion criteria considered patients aged at least 18 years old and less than 70 years old with active RA (defined as at least one swollen joint, erythrocyte sedimentation rate over 20 mm/h, or C-reactive protein more than 20 mg/dl). Patients were not receiving nonsteroidal anti-inflammatory drugs (NSAIDs) except by acetaminophen during the 2 weeks before receiving the first dose of the test drug. Patients were allowed to take prednisone or chloroquine (stable doses). MTX was administered to all patients as standard treatment. This study was approved by the Research Ethics Committee of the Valdivia Heath Service. A written and signed informed consent was obtained from all patients printed in their native language (Spanish) at the beginning of the study.

Patients with other non-degenerative diseases or other joint diseases that could interfere with RA evaluation (i.e., gout, condrocalcinosis, psoriatic arthritis, infectious arthritis, reactive, or spondilitic arthritis), women with child-bearing

potential, pregnant, or breast-feeding, and patients with severely limiting arthritis that renders patient subject to surgery or severely crippling or prostrated patients were excluded. Other exclusion criteria were the following: the use of intra-articular steroids during the month before enrollment; concomitant treatment with hydantoin, lithium, or anticoagulant drugs; history of peptic ulcer or gastrointestinal bleeding during 6 months before the study; history of hypersensitivity or adverse effects to NSAIDs; renal failure; hepatic failure; severe heart failure; hematologic diseases; history of alcohol or drug abuse; patients who participated in any other research since 1 month before enrollment; and patients not willing to attend regular checkup visits as agreed in the study period. All data were recollected from Hospital Regional of Valdivia and Osorno, Chile.

Interventions

Each patient considered eligible for the study was randomized by a computer code to one of two treatment arms:

Group 1 (active) received coated blue tablets containing 30 mg of andrographolides three times a day, in the morning, afternoon, and night. Group 2 (placebo) received coated blue tablets containing starch in the same way.

The herbal medicine intervention used in this trial was a highly purified composition standardized dried extract of *A. paniculata* (Burm. f.) Wall ex Nees (Acanthaceae). The product used was indexed as FANG(30) tablets, made from a dried extract of *A. paniculata*, manufactured by Farmindustria S.A. laboratories (Santiago, Chile) according to good manufacturing practice guidelines. The tablets contain lactose SD, avicel PH102, starch glycolate, talc, and magnesium stearate. The product is also registered at Institute of Public Health of Chile as an anti-inflammatory drug currently used for the upper respiratory tract infections.

The drug was kept according to the instructions of the manufacturer and separated from normal stocks of the hospital. The duration of the treatment was 14 weeks, excluding a 2-week washout period.

Characteristics of the herbal product were as follows. The extract was obtained from leaves and aerial parts of *A. paniculata* (Paractin®) that was kindly provided by Herbal Powers S.A. (Miami, USA). The herbal medicine intervention was a standardized composition of this dried extract of *A. paniculata*. The solvent used in the extract was alcohol (75% ethanol), and the ratio of herbal drug to extract was 10:1. A staff botanist visually identified the growing plant. The lot number for the *A. paniculata* extract used in this study was PAR-070801-2.

A voucher specimen was retained (no. 20050520) and was kept at Herbal Powers S.A. Each tablet contained 100 mg of the extract. During 14 weeks, tablets before meals three times per day were given. This dosage regimen was determined in previous clinical trials testing the effects of similar *A. paniculata* extracts [28, 29]. The percentages of quantified chemical constituents per tablet was as follows: 30 mg of total andrographolides (30% *w*/*w*), which comprises approximately 3% *w*/*w* of 14-deoxyandrographolide and 0.2% *w*/*w* of neoandrographolide.

A high-pressure liquid chromatography chemical fingerprint for the extract of *A. paniculata* was performed. The method of analysis was as follows: The compounds were extracted with acetone (4:1) and then analyzed by high-performance liquid chromatography (HPLC) using a reverse-phase RP-C18 licrospher column (4×125 mm). The mobile phase consisted of acetonitrile 26% and phosphoric acid 0.5%, at a rate of 1.1 ml/min, using a wavelength of 228 nm according to Burgos et al. [30]. The analysis was done by an analyst with 10 years experience in the methods at an independent laboratory (Indena SpA, Milano, Italy). The product sample is also kept at the Laboratory of Toxicology. The following reference standards were used: andrographolide (98%) purchased from Sigma (St. Louis, MO) and 14-deoxyandrographolide (90%) and neoandrographolide (90%) supplied by Indena SpA (Milano, Italy). The purity of these reference standards was assumed as provided by the suppliers. The placebo tablets used in this trial were identical in size (lactose powder filling) and color (with food coloring) to *A. paniculata* tablets.

During the study, no NSAIDs were allowed. Nonetheless, consent for the use of other NSAIDs was obtained from the patients since these are non-prescription medicines in Chile, and this information was included in the analysis. Exercise and/or physiotherapy were allowed. Paracetamol was used as a rescue drug in patients with episodes of severe pain.

The researcher distributed the test product and placebo only to those patients included in the protocol and that followed the fixed procedures. The researcher confirmed in writing the reception of the test products. Leftover medicines (study drug and placebo tablets) were returned once the study was complete.

Objectives

We hypothesized that *A. paniculata* tablets containing 30% andrographolide reduces clinical signs and symptoms of pain and swelling parameters evaluated by a visual analog pain scale (VAPS) in RA patients.

The main objective of the study was to evaluate the efficacy of these *A. paniculata* tablets to diminish the level of joint pain, including stiffness, impaired activity, swollen joint, and tiredness, in RA female patients after 14 weeks of treatment, as compared with a placebo group. Secondary

objectives were to assess the effect of this standardized extract of *A. paniculata* over immunological parameters associated with inflammatory processes. In addition, using health standardized questionnaires, safety and tolerability were evaluated.

Outcomes

Primary outcomes

Reduction of joints pain, stiffness, and tiredness symptoms were assessed by a VAPS of 0–10 cm (0 cm means not symptoms and 10 cm means maximal severity effect). To assess the number of joints with pain and swollen, a scale of 0 to 68 points was used (0, no joints affected and 68, all evaluated joints affected, according to ACR [31]). Impaired activity was evaluated using the European League Against Rheumatism response criteria (EULAR) by total grade of joints with swelling and tenderness signs from 0 to 204 points; 0 represents no joints affected and 204 means the maximum cumulative count of all evaluated joint's individual degree of swelling (scale 0=no pain, 1=patient complain of pain, 2=patient complains of pain and winces, 3=patient complains of pain, winces and withdraws) [31]. Finally, duration of morning stiffness was evaluated in hours. All clinical parameters were assessed at baseline, at the end of week 2, and in advance every 4 weeks until 14 weeks of treatment were completed.

Secondary outcomes

Other variables of efficacy in the treatment of RA assessed in the study were immunological parameters and Health Assessment Quality (HAQ) and Short Form Health Survey (SF36) standardized health questionnaires [33]. All these surveys were evaluated at baseline, at the end of week 2, and then every 4 weeks until the end of the study. The levels of rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein were determined at baseline, 6, and 14 weeks. At day 0, week 6, and week 14, complete blood counts including leukocytes and platelets counting, hematocrit, and hemoglobin were conducted. At that time, the following immunological markers were also assessed: antinuclear antibodies (ANA), anti-car-diolipin antibodies (ACA), extractable nuclear antigens antibodies (ENA), serum immunoglobulins (IgG, IgA, IgM, ACA-IgG, and ACA-IgM), and complement components C3 and C4.

Other parameters assessed

Chest, hands, and feet X-ray plates were also done at day 0 and at end of the study (week 14).

Confounders

Data regarding the type and frequency of the intake of all drugs and the use of other medicines, as possible confounders, were collected along with the VAPS pain intensity in the protocol sheets. Furthermore, possible confounder covariates (age, NSAIDs consumption, and years with RA clinically diagnosed) were considered and controlled in statistical analysis.

Adverse reactions or events

At weeks 0, 6, and 14 (end of treatment), a total of 20 ml of venous blood was obtained for safety evaluation. Glucose, albumin, cholesterol, calcium, phosphorus, creatinkinase (CK), lactate dehydrogenase (LDH), creatinine, blood urea nitrogen (BUN), uric acid, aspartate aminotransferase (AST), alkaline phosphatase, and alanine aminotransferase (ALT), and bilirubin were evaluated at these times. Possible adverse reactions were assessed by rheumatologists at each visit.

Sample size

We used the following assumptions to estimate the necessary sample size. A 2.0 cm was considered the smallest average difference change between active drug (AD) and placebo treatments to detect a clinical effect in a VAPS of 10 cm (equivalent to 20%) [34, 35]. The power of 90% was used to detect a true difference in outcome between the placebo and the intervention arm. The level of significance was set at 5%, and two sided was used to reject the null hypothesis. The common standard deviation used in the sample size was 1.7 cm. The sample size determined to each group was 18 subjects. We used the compliance adjustment formula to adjusted n per arm equals $N/([c_1+c_2-1]^2)$, assuming a 90% in each group, estimating 27 subjects per group. nQuery Advisor™ version 4.0 software was used to calculate sample size.

Randomization—sequence generation

The 60 RA women were allocated in two groups. For each one, random codes with a permuted randomization scheme were generated by computer.

Randomization—allocation concealment

Participants were not with informed their group assignment code. The study physicians did not share their own examination results, did not handle the study products, and

did not know the assigned treatment. Two envelopes contained each individual's treatment assignment; one set was for the Rheumatology Unit to keep for emergency care and the other was kept with the Principal Investigator. The two envelopes remained sealed until data analysis.

Randomization—implementation

All appointments for the 62 participants were arranged at the Rheumatology Unit of each hospital. Sixty patients fulfilled the inclusion criteria. Finally, 60 included patients were randomized into active or placebo groups based on the order of their chosen date and the arrival time for their post-screening clinic. A total of 30 placebo and 30 active patients, respectively, were allocated to each group, according to the computer generated allocation sequence by the study statistician.

Blinding

The appearances of the test product and placebo-coated tablets were identical, and no aroma was detected from either. Achievement of blindness was validated before the trial in a group of 15 volunteers. At the completion of treatment, we conducted a simple survey asking the participants to guess whether they were in the study product or placebo group in order to evaluate the extent of study blindness.

Statistical analysis

We performed an exploratory analysis for all dataset and separately to each treatment group. Descriptive statistics and 95% CIs were used to estimate patient parameters. To evaluate normality assumption, we used Shapiro–Wilks test, considering non-normal distribution, those presenting p values lower than 0.05. The majority of the variables did not present normal distribution; therefore, a Kruskal–Wallis non-parametric test was used to compare baseline data between treatments for hematological and biochemical determination, health standard questionnaires, and VAPS. To evaluate the degree of independence between repeated measures during the period of study, the Pearson correlation test was used. We observed a moderate to high intra-individual correlation to all outcome variables over repeated measurements (data no shown); therefore, we used generalized estimating equations (GEE) to evaluate these data considering an exchangeable correlation structure using robust standard errors [36]. GEE analysis is a quasi-likelihood iterative procedure to estimate a marginal average regression coefficient in longitudinal studies with repeated measures, which takes into account the data correlation structure, missing data, and several time-dependent and time-independent covariates [37–39].

Three models were fitted to each outcome variable: the first model fitted, considered group variable (0=placebo; 1=AD) plus interaction variable (group×time); this model was adjusted by baseline covariates such as age, NSAIDs consumption, and years after diagnosis of RA. In all adjusted models, the interaction terms were non-significant, indicating that the treatment effect on the outcome does not vary differently over time among groups (data no shown); therefore, we fitted a simple model (model 1). In this last model, the group variable was not statistically significant, indicating non-differences between AD and placebo. Finally, we performed two models (models 2a and 2b) considering only time variable for each treatment group adjusting by covariates previously defined.

Model 1: Simple model

$$Y_{it} = \beta_0 + \beta_1\ \text{group} + \beta_2\ \text{time} + \beta_3\ \text{age} + \beta_4\ \text{NSAID consumption} + \beta_5\ \text{years with AR} + [\text{corr}] + \varepsilon_{it}$$

Model 2: Time variable

Active drug (Model 2a)

$$\text{2a; } Y_{it} = \beta_0 + \beta_1\ \text{time} + \beta_2\ \text{age} + + \beta_3\ \text{NSAID consumption} + \beta_4\ \text{years with AR} + [\text{corr}] + \varepsilon_{it}$$

Placebo (Model 2b)

$$\text{2b; } Y_{it} = \beta_0 + \beta_1\ \text{time} + \beta_2\ \text{age} + \beta_3\ \text{NSAID consumption} + \beta_4\ \text{years with AR} + [\text{corr}] + \varepsilon_{it}$$

where Y_{it} are the outcomes for some subjects i at time t, β_0 is the intercept, β_1 to β_n are regression coefficients for each independent exposure (treatment group) and covariates, corr (working correlation structure), and ε_{it} is the error for subject i at time t. The xtgee procedure of STATA was used to evaluate these models [40]. All analyses were done based on intention to treat. Graphical methods were used to show VAPS changes over time (weeks). In all models, time variable was used as a continuous variable.

Results

Participant flow

Figure 1 summarizes the enrollment, treatment allocation, and data analysis for the 60 patients with primary RA.

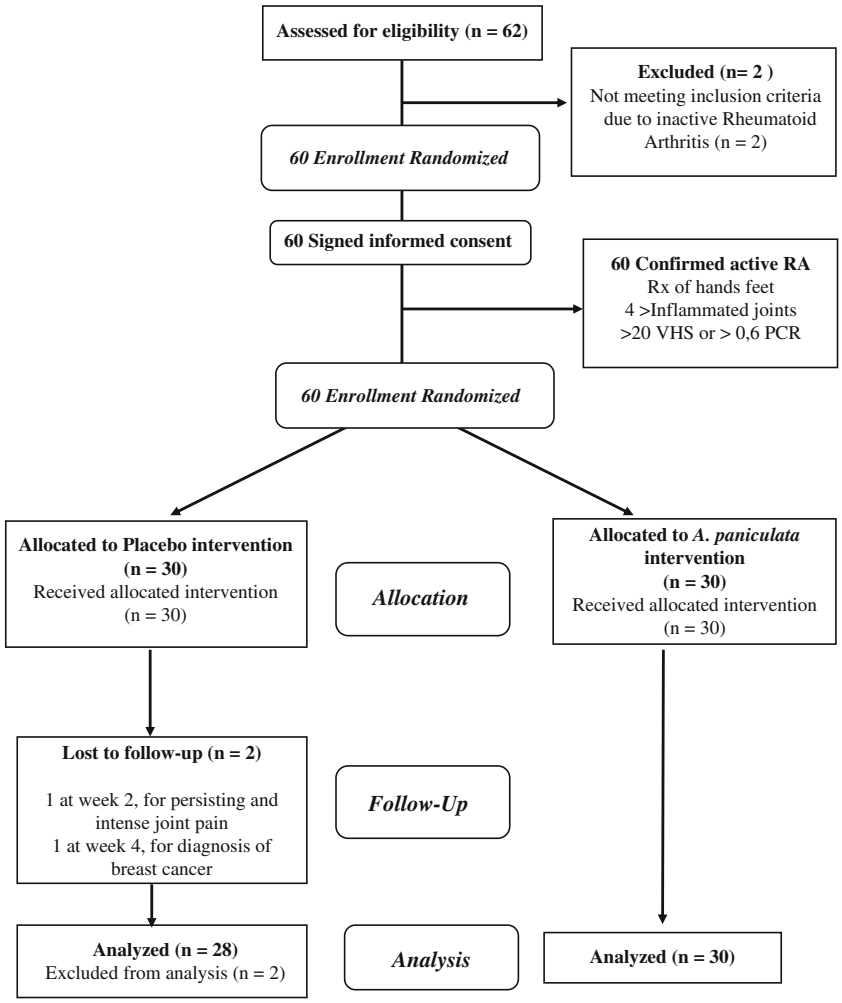


Fig. 1 Flowchart of the study

Recruitment

Table 1 shows the time periods of recruitment, washout, baseline, treatment, and follow-up phases. The trial lasted 12 months starting from 10/07/2006 to 10/14/2007. Study was ended when planned recruitment was completed.

Baseline data

Table 2 describes the demographic characteristic of both groups. Patients in both groups were comparable in age, weight, sex, years with RA, body mass index, and intake of NSAIDs. Primary VAPS variables and secondary immunological outcomes were similar in both groups. Table 3 shows the baseline data of disease parameters assessed in both groups.

Numbers analyzed

Two patients failed to comply with treatment resulting in 58 of 60 (96.7%) compliance rate. The number of participants analyzed at baseline and treatment term for placebo at week

Table 1 Time periods of trial progression

Phase	Activity	Dates (month/day/year)
Recruitment	Assessment of eligibility	10/07/2006 to 8/30/2007
Washout	Informed consent; collection Medical screening visits	11/07/2006 to 9/30/2007
Baseline	Pretreatment clinic visits w0	12/02/2006 to 9/31/2007
Treatment	Visits: w2, w6, w10 and w14	12/16/2006 to 10/14/2007
Follow-up	Post treatment clinic visit Examination	01/04/2008 to 03/07/2008

Table 2 Demographic characteristics of patients at day "0"

	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 RA diagnosed	6.48 (0.66–22.34)	6.69 (0.66–25.15)
BMI (kg/m^2)	30.00 (19.70–41.40)	29.20 (18.30–44.50)
Height (m)	1.52 (1.30–1.75)	1.51 (1.38–1.69)
Weight (kg)	69.93 (43.00–106.00)	67.15 (39.50–100.00)
Intake of NSAIDs, *n* (%)	17 (60.71)	18 (60.00)

BMI body max index, *RA* rheumatoid arthritis, *NSAIDs* Non-steroidal anti-inflammatory drugs

"0" was 30 and week "2" 29 and for weeks "6," "10," and "14" 28 patients. On the other hand, in the active group, the number of patients analyzed at weeks "0," "2," "6," "10," and "14" was 30.

Main outcome measure(s)

VAPS was our main outcome. We fitted a GEE model to evaluate the relationship between AD versus placebo (model 1) and one to AD drug (model 2a) and one to placebo group (model 2b), respectively, adjusting for explanatory variables using an exchangeable working correlation structure (Table 4).

In general, model 1 show that *treatment* (AD vs placebo) variable did not have a significant association with any outcome variables for VAPS and HAQ and SF36 health questionnaires during the study period. However, regression coefficients (slopes) to all predictive variables trend in negative direction, indicating a decrease in the average values for every outcome adjusted variables by age, NSAIDs consumption, and number of years with RA.

When we fitted each treatment separately and adjusted for the same covariates, we observed a negative and significant association in receivers of AD (model 2a) for tender joints −0.13 95% CI (−0.22 to 0.06; p=0.001), number of swollen joints −0.15 95%CI (−0.29 to −0.02; p= 0.02), total grade of swollen joints −0.27 95%CI (−0.48 to −0.07; p=0.010), number of tender joints −0.25 95%CI (−0.48 to −0.02; p=0.033), total grade of tender joints −0.47 95%CI (−0.77 to −0.17;p=0.002) and HAQ −0.52 95%CI (−0.82 to -0.21; p<0.001) and SF36 health questionnaires +0,02 95%CI (0.01 to 0.02; p<0.001), indicating that in average these signs and symptoms diminished significantly during the study period (Table 4). The placebo model 2b shows a significant negative association only for numbers and total grade of swollen joints, HQA, SF36 health questionnaire (Table 4).

The average response profile of RA patients to VAPS and health questionnaires are shown in Fig. 2 for each outcome variable. We can observe that the effect on pain and swollen of joints diminished faster over time within AD group, as compared to placebo group, in which the effect on outcome variables was relatively constant over time.

The biochemical parameters (Table 5) had a significantly negative association in model 2a for total protein −0.01 95%CI (−0.0002 to −0.018; p=0.021) and RF −0.68 95% CI (−1.19 to −0.16; p=0.010), indicating a decrease of these parameters during the period of study, being this attributed to the effect of AD. A significantly positive association was observed for calcium +0.02 95%CI (0.002 to 0.03; p=0.02). To hematological parameters, only hemoglobin −0.03 95%CI (−0.04 to −0.01; p=0.004) was statistically significant over AD treatment (Table 6). To immunological parameters (Table 7), we observed a significant and positive effect to c_4 +4.41 95%CI (0.39 to 8.44; p= 0.031; model 1). To model 2a (AD), IgA −1.5 95%CI (−2.98 to −0.03; p=0.046), IgM −0.73 95%CI (−1.28 to 0.19; p=<0.008), and c_4 −0.16 95%CI (−0.26 to −0.06; p=0.002) shown a negative association indicating a strong diminishing effect of this outcome variables over the time within the AD treatment. For the placebo group (model 2b), only a significantly negative effect was observed for ENA −0.01 95%CI (−0.02 to −0.001; p= 0.027) and hemoglobin −0.04 95%CI (−0.06 to −0.02; p= 0.001; Table 6).

Metabolic parameters such as appetite, weight, liver, and kidney functions, along with hemodynamic and hematological parameters remained stable over time and did not show any difference in both groups.

Chest radiological examination did not show pulmonary lesions, and findings in hands and feet images remained compatible with RA diagnosis.

Ancillary analyses

Response rate to VAPS and health questionnaires

The percentage (%) and absolute number of responder patients (n) to VAPS during the period of treatment were the following: at the beginning for both treatments, a 100.0%; week 2, 96.67% (29) for placebo and 100% (30) AD; and weeks 6 to 14, 93.33% (28) and 100.0%(30) for

Table 3 Hematological, biochemical, immunological parameters, VAPS, and health questionnaires at baseline for active drug and placebo group, respectively

Hematological and biochemical determinations	Active drug (n=30)			Placebo (n=28)		
	Mean	Median	SD	Mean	Median	SD
Rheumatoid factor (UI/ml)	111.16	119.00	67.16	117.94	130.00	75.20
PCR (mg/dl)	2.13	0.89	4.02	1.41	0.90	1.46
Glucose (mg/dl)	95.93	91.00	19.57	100.88	91.00	29.98
Creatinine (mg/dl)	0.73	0.73	0.14	0.70	0.70	0.15
Calcium (mg/dl)	9.18	9.10	0.33	9.23	9.20	0.45
BUN (mg/dl)	15.66	16.10	4.42	21.41	15.65	28.56
Creatine kinase (UI/l)	89.28	75.50	45.58	79.36	65.00	56.75
Albumin (g/dl)	4.24	4.30	0.39	4.37	4.40	0.31
Cholesterol (mg/dl)	206.19	207.00	46.95	219.04	217.00	54.25
LDH	205.57	205.00	37.69	190.92	189.00	38.31
Bilirubin (mg/dl)	0.44	0.49	0.28	0.46	0.44	0.17
FA (UI/l)	100.78	90.00	38.94	99.28	90.00	32.42
Leukocytes, $x10^3$/ul	8.77	8.30	2.54	9.43	9.02	2.06
Platelets, $x10^3$/ul	291.67	296.00	76.04	308.32	306.00	67.46
Hemoglobin (g/dl)	13.22	13.20	0.93	13.64	14.00	2.12
Hematocrit (%)	39.97	39.70	2.35	41.35	40.20	6.13
AST (UI/l)	21.90	21.00	7.16	23.96	20.00	12.27
ALT (UI/l)	24.81	22.00	10.88	24.24	18.00	15.07
ANA, titer	94.54	80.00	116.54	67.20	74.58	40.00
ENA (0=negative, 1=positive)	0.12	0.00	0.33	0.24	0.00	0.43
IgA (mg/dl)	315.69	293.70	133.73	339.28	335.60	143.12
IgG (mg/dl)	1160.46	1122.00	288.77	1227.34	297.14	1261.30
IgM (mg/dl)	151.64	140.50	55.58	146.71	136.00	66.74
C3 (mg/dl)	138.13	135.50	22.23	138.83	132.90	20.83
C4 (mg/dl)	33.36	32.10	8.85	30.55	30.10	8.41
ACA-IGM mpl	27.01	20.00	15.04	28.79	19.00	18.40
ACA- IGG gpl	28.93	19.00	16.89	27.26	20.50	16.55
ACR parameters						
Number of swollen joints (0–66)	10.93	9.00	6.63	12.76	13.00	6.65
Number of tender joints (0–68)	17.15	13.00	13.25	21.04	13.00	17.19
Pain (0–100 mm) VAPS	6.67	7.10	2.58	5.53	5.70	2.97
HAQ (0–64)	21.24	19.00	13.73	22.72	24.00	14.05
ESR (mm/h)	31.21	29.00	20.54	21.52	19.00	11.19
Other clinical parameters						
Total grade of swollen joints (0–204)	14.39	11.00	12.25	17.12	16.00	11.08
Total grade of tender joints (0–204)	21.27	14.00	18.08	25.40	17.00	21.29
Stiffness (hours)	0.82	0.33	0.96	0.94	0.17	1.75
Tiredness (0–100 mm) VAS	4.75	5.00	2.82	4.04	4.00	2.92
SF 36 (1–4)	2.43	2.30	0.35	2.53	2.50	0.33

ESR erythrocyte sedimentation rate, *PCR* protein–C-reactive, *BUN* blood urea nitrogen, *FA* fatty acid; *AST* aspartate amino transferase, *ALT* alaline aminitransferase, *ANA* antinuclear antibody, *ENA* extractable nuclear antigen, *HAQ* Health Assessment Questionnaire, *SF36* Short Form of Health Questionnaire

Table 4 Result of the GEE analysis with VAPS, ACR, EULAR, and SF36 as outcome variables and treatment (AD vs placebo) and active drug (AD) and placebo, respectively

Variable	Regression coefficient	Standard error	*p* value	CI (95%)	
				Lower	Upper
Tender joints					
Model 1: Treatment (AD vs P)[a]	0.96	0.68	0.161	−0.38	2.29
Model 2a: active Drug[b]	−0.13	0.04	0.001	−0.22	−0.06
Model 2b: Placebo[b]	0.05	0.04	0.254	−0.03	0.12
Joints stiffness					
Model 1: Treatment (AD vs P)[a]	−0.32	0.24	0.194	−0.81	0.16
Model 2a: Active drug[b]	0.01	0.01	0.792	−0.02	0.03
Model 2b: Placebo[b]	−0.02	0.02	0.295	−0.05	0.02
Tiredness					
Model 1: Treatment (AD vs P)[a]	0.12	0.78	0.868	−1.40	1.65
Model 2a: Active drug[b]	−0.07	0.04	0.093	−0.15	0.01
Model 2b: Placebo[b]	−0.01	0.04	0.845	−0.08	0.63
Number of swollen joints					
Model 1: Treatment (AD vs P)[a]	−1.84	1.77	0.297	−5.30	1.62
Model 2a: Active Drug[b]	−0.15	0.07	0.020	−0.29	−0.02
Model 2b: Placebo[b]	−0.27	0.08	0.001	−0.43	−0.11
Total grade of swollen joints					
Model 1: Treatment (AD vs P)[a]	−1.96	2.66	0.463	−7.18	3.26
Model 2a: Active Drug[b]	−0.27	0.11	0.010	−0.48	−0.07
Model 2b: Placebo[b]	−0.33	0.12	0.006	−0.57	−0.10
Number of tender joints					
Model 1: Treatment (AD vs P)[a]	0.06	3.86	0.987	−7.50	7.62
Model 2a: Active Drug[b]	−0.25	0.12	0.033	−0.48	−0.02
Model 2b: Placebo[b]	−0.19	0.11	0.099	−0.41	0.03
Total grade of tender joints					
Model 1: Treatment (AD vs P)[a]	0.96	4.51	0.831	−7.87	0.98
Model 2a: Active Drug[b]	−0.47	0.15	0.002	−0.77	−0.17
Model 2b: Placebo[b]	−0.22	0.16	0.167	−0.52	0.09
Health Assessment Questionnaire (HAQ)					
Model 1: Treatment (AD vs P)[a]	−1.78	3.50	0.611	−8.64	5.08
Model 2a: Active Drug[b]	−0.52	0.16	<0.001	−0.82	−0.21
Model 2b: Placebo[b]	−0.56	0.16	<0.001	−0.87	−0.25
SF36 Health Questionnaire					
Model 1: Treatment (AD vs P)[a]	−0.12	0.10	0.244	−0.32	0.08
Model 2a: Active Drug[b]	0.02	0.00	<0.001	0.01	0.02
Model 2b: Placebo[b]	0.01	0.00	<0.001	0.00	0.02

Model adjusted for age, NSAIDs consumption, and number of years with rheumatoid arthritis

[a] Intergroup

[b] Intragroup

CI confidence interval, *AD* active drug, *P* placebo, *SF36* Short Form Health Questionnaire

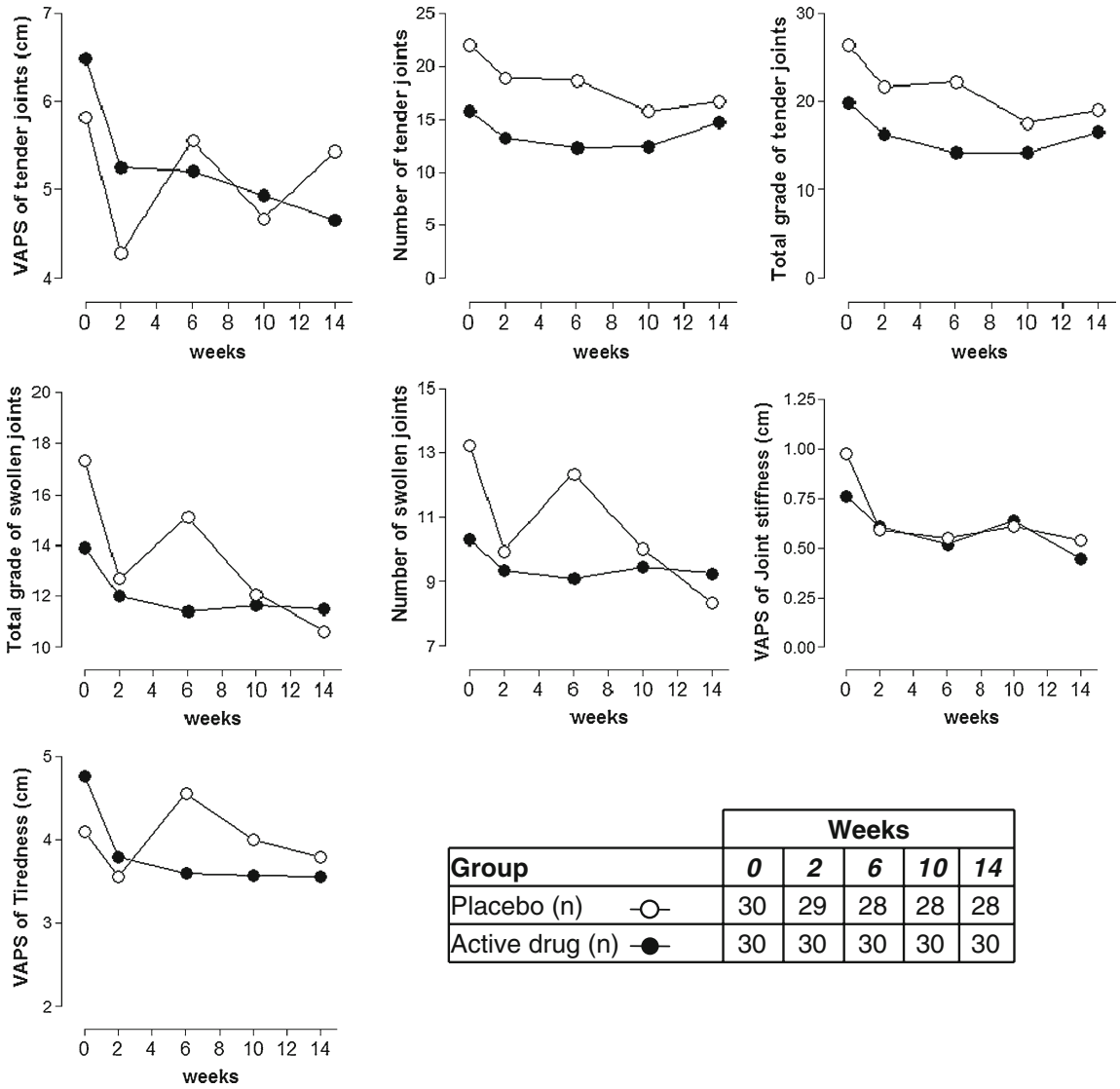
placebo and AD, respectively. In the case of health questionnaires, the percentage of response was 100% for both treated groups at the beginning, 93.33% (28) and 100.0% (30) at week 6, respectively, and 89.28%(25) and 100.0% (30) at week 14, respectively.

Adverse effects

In the placebo group, two patients reported symptoms of nausea, one diarrhea, two stomach discomfort, one dizziness, two tiredness, and one headache. In the active treatment group, three patients reported headache, one diarrhea, two nausea, one stomach discomfort, one fatigue, one common cold, one pruritus/rash, and one cramps.

Discussion

A. paniculata has shown efficacy in the treatment of common colds with doses of 1,200 mg/day by using a dried extract standardized to 5% of andrographolide (60 mg of andrographolide/day). The drug reduced significantly the



Group	Weeks 0	2	6	10	14
Placebo (n)	30	29	28	28	28
Active drug (n)	30	30	30	30	30

Fig. 2 VAPS, outcome scores of tender joints, number of tender joints, grade of tender joints, joint stiffness, grade of swollen joints, number of swollen joints and tiredness, over time by active drug and placebo groups. Each point represents the arithmetic mean. In the table, the number of patients at risk per group over time is depicted

intensity of symptoms such as tiredness, sleeplessness, sore throat, and nasal secretion [28]. Its clinical efficacy has been associated to the anti-inflammatory properties [8]. Moreover, andrographolide, the main diterpenic labdane, is a potent inhibitor of NF-κB [12], a transcription factor linked to pro-inflammatory expression, such as COX-2, iNOS, and TNF-α [41, 42]. Since NF-κB is involved in the pathogenesis of RA [43], we hypothesized that an *A. paniculata* tablet (30% of andrographolide) can reduce the joint pain in patients suffering RA. In this sense, 60 patients were enrolled and divided in active and placebo group in this prospective, randomized, double-blind study. Several clinical parameters of pain and swollen joints were evaluated. Comparison between placebo and active group did not show significant differences over evaluated outcomes during the treatment period. However, when each treatment was evaluated separately, a clear and significant tendency to decrease the VAPS main outcomes for the AD group was observed. A significant reduction for tender joints, number of swollen joints, total grade of swollen joints, number of tender joints, total grade of tender joints, and HAQ and SF36 health questionnaires was found. In the placebo group, only the numbers and total grade of swollen joints and HQA, SF36, and health questionnaire showed a negative association. The latter finding could indicate a therapeutic effect of MTX on RA patients. In this sense, several reports describe a reduction of swollen joint count between 6 and 18 weeks of MTX treatment [44].

Our finding suggests that *A. paniculata* formulation may have an additional therapeutical effect over MTX in

Table 5 Result of the GEE analysis with biochemical parameters as outcome variables and treatment (AD vs placebo) and active drug (AD) and placebo, respectively

Variable	Regression coefficient	SE	*p* value	95% confidence interval	
Rheumatoid factor (UI/ml)					
Model 1: Treatment (AD vs P)[a]	5.05	18.77	0.788	−31.73	41.84
Model 2a: Active drug[b]	−0.68	0.26	0.010	−1.19	−0.16
Model 2b: P	−0.19	0.29	0.527	−0.76	0.39
PCR (mg/dl)					
Model 1: Treatment (AD vs P)[a]	0.16	0.58	0.785	−0.98	1.30
Model 2a: Active drug[b]	−0.03	0.03	0.369	−0.10	0.04
Model 2b: P[b]	−0.04	0.02	0.134	−0.08	0.01
Glucose (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−9.46	6.77	0.162	−22.73	3.80
Model 2a: Active drug[b]	−0.09	0.21	0.672	−0.50	0.32
Model 2b: P[b]	−0.13	0.29	0.655	−0.69	0.43
Creatinine (mg/dl)					
Model 1: Treatment (AD vs P)[a]	0.04	0.03	0.211	−0.02	0.11
Model 2a: Active drug[b]	−0.002	0.002	0.154	−0.01	0.001
Model 2b: P[b]	−0.002	0.002	0.339	−0.01	0.002
Total protein (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−0.14	0.13	0.294	−0.41	0.12
Model 2a: Active drug[b]	−0.01	0.006	0.021	−0.0002	−0.018
Model 2b: P[b]	−0.004	0.008	0.561	−0.01	0.02
Calcium (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−0.08	0.11	0.449	−0.30	0.13
Model 2a: Active drug[b]	0.02	0.01	0.021	0.002	0.03
Model 2b: P[b]	−0.01	0.01	0.551	−0.02	0.01
BUN (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−5.16	3.49	0.139	−11.99	1.67
Model 2a: Active drug[b]	0.002	0.05	0.967	−0.10	0.10
Model 2b: P[b]	−0.45	0.39	0.248	−1.21	0.31
Creatine kinase (UI/l)					
Model 1: Treatment (AD vs P)[a]	5.27	10.29	0.609	−14.91	25.45
Model 2a: Active drug[b]	−0.90	0.47	0.055	−1.83	0.01
Model 2b: P[b]	0.10	0.72	0.879	−1.30	1.52
LDH (UI/l)					
Model 1: Treatment (AD vs P)[a]	17.68	10.88	0.104	−3.65	39.01
Model 2a: Active Drug[b]	0.09	0.49	0.862	−0.88	1.05
Model 2b: PLACEBO[b]	0.28	0.31	0.370	−0.33	0.88
Albumin (g/dl)					
Model 1: Treatment (AD vs P)[a]	−0.09	0.09	0.325	−0.26	0.08
Model 2a: Active drug[b]	0.001	0.005	0.840	−0.01	0.01
Model 2b: P[b]	0.004	0.005	0.343	−0.005	0.01
Cholesterol (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−4.50	12.78	0.725	−29.55	20.55
Model 2a: Active drug[b]	−0.11	0.56	0.843	−1.21	0.99
Model 2b: P[b]	−0.75	0.62	0.229	−1.98	0.47
Bilirubin (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−0.27	1.33	0.840	−2.87	2.33
Model 2a: Active drug[b]	0.20	0.15	0.163	−0.08	0.49
Model 2b: P[b]	0.004	0.003	0.183	−0.002	0.01

Table 5 (continued)

Variable	Regression coefficient	SE	*p* value	95% confidence interval	
FA (UI/l)					
Model 1: Treatment (AD vs P)[a]	−1.80	9.31	0.847	−20.03	16.44
Model 2a: Active drug[b]	−0.21	0.20	0.295	−0.60	0.18
Model 2b: P[b]	−0.08	0.18	0.643	−0.43	0.26
AST (UI/l)					
Model 1: Treatment (AD vs P)[a]	−2.56	2.27	0.258	−7.00	1.88
Model 2a: Active drug[b]	0.07	0.11	0.535	−0.15	0.28
Model 2b: P[b]	−0.10	0.18	0.587	−0.46	0.26
ALT (UI/l)					
Model 1: Treatment (AD vs P)[a]	−0.36	3.23	0.910	−6.69	5.96
Model 2a: Active drug[b]	0.03	0.11	0.767	−0.19	0.25
Model 2b: P[b]	0.17	0.10	0.091	−0.03	0.38

Model adjusted for age, NSAIDs consumption, and number of years with rheumatoid arthritis

[a] Intergroup

[b] Intragroup

PCR protein–C-reactive, *BUN* blood urea nitrogen, *FA* fatty acid, *AST* Aspartate aminotransferase, *ALT* alaline aminotransferase, *AD* active drug, *P* placebo

reducing pain and inflammatory clinical symptoms during treatment period. The beneficial effect on pain and inflammatory symptoms with the *A. paniculata* formulation could be associated to andrographolide standardization considering its ability to inhibit NF-κB binding to DNA [12, 45]. This is closely associated with the inhibition of COX-2 [12] and the reduction of PGE2 production [13], one of the main mechanisms for the control of inflammation and pain in RA by NSAIDs [46]. The dose of andrographolide used in the present study was 1.25 mg kg^{-1} day^{-1}. It has been reported that 1 mg kg^{-1} day^{-1} reaches a steady state plasma concentration of 1.9 μM [29], a concentration able to reduce the PGE2 production [13].

Table 6 Result of the GEE analysis with hematological parameters as outcome variables and treatment active drug vs placebo; active drug and placebo, respectively

Variable	Regression coefficient	SE	*p* value	95% Confidence interval	
ESR (mm/h)					
Model 1: Treatment (AD vs P)[a]	4.11	4.31	0.340	−4.33	12.56
Model 2a: Active drug[b]	0.04	0.18	0.830	−0.31	0.38
Model 2b: P[b]	0.01	0.13	0.949	−0.25	0.27
Leukocytes, $x10^3$/ul					
Model 1: Treatment (AD vs P)[a]	−0.62	0.74	0.399	−2.07	0.83
Model 2a: Active drug[b]	−0.04	0.04	0.308	−0.12	0.04
Model 2b: P[b]	−0.04	0.03	0.149	−0.10	0.01
Platelets, $x10^3$/ul					
Model 1: Treatment (AD vs P)[a]	−22.69	20.90	0.278	−63.66	18.28
Model 2a: Active drug[b]	0.32	0.49	0.517	−0.64	1.27
Model 2b: P[b]	−0.15	0.82	0.857	−1.76	1.46
Hemoglobin (g/dl)					
Model 1: Treatment (AD vs P)[a]	−0.31	0.46	0.501	−1.21	0.59
Model 2a: Active drug[b]	−0.03	0.01	0.004	−0.04	−0.01
Model 2b: P[b]	−0.04	0.01	0.001	−0.06	−0.02
Hematocrit (%)					
Model 1: Treatment (AD vs P)[a]	−0.87	1.26	0.490	−3.35	1.60
Model 2a: Active drug[b]	−0.03	0.03	0.196	−0.09	0.02
Model 2b: P[b]	−0.05	0.03	0.096	−0.11	0.01

Model adjusted for age, NSAIDs consumption, and number of years with rheumatoid arthritis

[a] Intergroup

[b] Intragroup

ESR erythrocyte sedimentation rate, *AD* active drug, *P* placebo

Table 7 Result of the GEE analysis with immunological parameters as outcome variables and treatment active drug vs placebo; active drug and placebo, respectively

Variable	Regression coefficient	SE	*p* value	95% Confidence interval	
ANA, titer					
Model 1: Treatment (AD vs P)[a]	20.28	23.81	0.394	−26.38	66.94
Model 2a: Active drug[b]	−1.13	1.47	0.443	−4.02	1.76
Model 2b: P[b]	0.24	1.18	0.843	−2.09	2.56
ENA (0=negative, 1=positive)					
Model 1: Treatment (AD vs P)[a]	−0.17	0.11	0.143	−0.39	0.06
Model 2a: Active drug[b]	0.01	0.004	0.121	−0.002	0.01
Model 2b: P[b]	−0.01	0.01	0.027	−0.02	−0.001
IgA (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−59.8	36.43	0.101	−131.21	11.6
Model 2a: Active drug[b]	−1.5	0.75	0.046	−2.98	−0.03
Model 2b: P[b]	−1.03	0.81	0.204	−2.61	0.56
IgG (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−109.41	76.27	0.151	−258.9	40.08
Model 2a: Active drug[b]	−3.27	3.62	0.367	−10.36	3.83
Model 2b: P[b]	−1.63	1.74	0.351	−5.04	1.79
IgM (mg/dl)					
Model 1: Treatment (AD vs P)[a]	14.05	17.7	0.427	−20.64	48.74
Model 2a: Active drug[b]	−0.73	0.28	0.008	−1.28	−0.19
Model 2b: P[b]	0.31	0.3	0.305	−0.28	0.91
C3 (mg/dl)					
Model 1: Treatment (AD vs P)[a]	−0.73	5.58	0.895	−10.21	11.69
Model 2a: Active drug[b]	0.39	0.21	0.06	−0.01	0.81
Model 2b: P[b]	0.24	0.22	0.28	−0.19	0.69
C4 (mg/dl)					
Model 1: Treatment (AD vs P)[a]	4.41	2.05	0.031	0.39	8.44
Model 2a: Active drug[b]	−0.16	0.05	0.002	−0.26	−0.06
Model 2b: P[b]	−0.1	0.06	0.107	−0.22	0.02
ACA-IGM mpl					
Model 1: Treatment (AD vs P)[a]	−2.11	4.84	0.663	−11.6	7.37
Model 2a: Active drug[b]	−0.37	0.25	0.145	−0.87	0.12
Model 2b: P[b]	−0.4	0.29	161	−0.97	0.16
ACA-IGG gpl					
Model 1: Treatment (AD vs P)[a]	−1.89	3.83	0.621	−9.4	5.61
Model 2a: Active drug[b]	0.43	0.25	0.082	−0.05	0.92
Model 2b: P[b]	−0.55	0.29	0.057	−1.12	0.01

Model adjusted for age, NSAIDs consumption, and number of years with rheumatoid arthritis

[a] Intergroup

[b] Intragroup

ACA anti-cardiolipin antibody, *ANA* antinuclear antibody, *ENA* extractable nuclear antibody, *AD* active drug, *P* placebo

Moreover, this effect is associated with a decrease of RF, creatine kinase, hemoglobin, IgA, and IgM. A correlation between RF titers and clinical disease activity has been reported widely [47–50]. RF titers decrease with medicines such as methotrexate, suggesting an indirect link with disease activity [47, 48, 51]. Andrographolide can reduce the TNF-α production in macrophages [14], an effect that could be associated with the reduction of auto-antibodies. It is known that a reduction of TNF-α can reduce significantly the RF levels [52]. The ability of andrographolide to

reduce antibody titer also has been demonstrated in experimental autoimmune encephalomyelitis as an inhibition of antibodies directed to myelin antigens [16]. A reduction of immunoglobin, 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 RA [49] and decrease of RF. Moreover, treatment with DMARDs reduces the level of IgM and IgA in patients affected with RA [51, 52] *A. paniculata* could be useful in decreasing the radiological progression in long-term treatments in RA patients. Moreover, we have recently demonstrated that andrographolide can reduce the NFAT activity, a transcription factor linked with bone erosion [23]. No significant differences in side effects between placebo and AD were observed. This indicates that the treatment was safe, non-toxic, and well tolerated. Only one patient reported pruritus. Although this effect could have been associated with the treatment, the literature describes allergic reactions and pruritus/rash with andrographolide or *A. paniculata* treatment [8]. In literature, side effects associated with *A. paniculata* or andrographolide, administered in much higher doses than the ones used in this study, have included 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[8]. In our study, nausea, diarrhea, and stomach discomfort was equally reported in both groups. Since methotrexate was given to all patients, these side effects could be more rather attributed to this drug and not to treatment with *A. paniculata*. In this sense, nausea and gastrointestinal distress is one of the most frequent adverse effects reported with methotrexate particularly when administered in association with prednisone in RA patients [44].

In overall, our results suggest that *A. paniculata* formulation (30% andrographolide) can reduce the symptoms and some RA immunological markers during a 14 weeks treatment, indicating a cumulative effect of the drug.

Generalizability

To assess whether *A. paniculata* formulation (30% andrographolide) has a clear therapeutic effect for RA in clinical medicine, a larger clinical trial would be needed. It will require a detailed sample size calculation covering different geographical areas, age, gender, and ethnic groups, or races groups for assessing generalizability. A longer treatment period should be tested in order to establish an optimal dose and length of treatment. Multiple batches of the product can also be included in determining the extent of the efficacy.

Overall evidence

We did not find the same distinct effect from *A. paniculata* treatment among 58 patients as from synthetic analgesics and DMARDs or a significant reduction of joint pain after a 14-week study period of treatment [7, 53]. A larger short-term study demonstrated that *A. paniculata* extract standardized to andrographolide, reduces the pain in the muscle, headache, earache, cough, and throat symptoms in uncomplicated upper respiratory tract infections [28, 54].

The effect of *A. paniculata* through the time suggests a reduction of pain and swelling and other clinical parameters associated to parameters such as RF and immunoglobulin. Since patients treated with *A. paniculata* formulation showed a significant tendency to improve the symptoms of RA, a larger and longer term study should demonstrate clearer differences in comparison with a placebo group.

The andrographolides present in the extract of *A. paniculata* such as andrographolide or neoandrographolide have been widely recognized as effective in reducing several inflammatory markers associated to RA disease, such as COX-2, iNOS, TNFα, IL-6 [12, 14, 55, 56]. Moreover, it has been demonstrated that andrographolide can reduce the PI3k pathway [18] and transcription factors associated with the pathogenesis of RA [12, 45]. We recently demonstrated that andrographolie interferes with NFAT activation in T cells and ERK1/2 and ERK5 phosphorylation [21]. The inhibitory effect of andrographolide on MAPK has been described [14, 15, 18], and this could be a relevant mechanism to explain the decrease of symptoms and signs and immunological parameters observed with the *A. paniculata* tablets, according to what has already been proposed for MAPKs, as potential therapeutic targets in RA [57].

Acknowledgments The authors would like to thank Herbal Powers (Miami, USA) for providing the *Andrographis paniculata* extract (PARACTIN®), Mr. Andrea Giori INDENA SpA (Milano, Italy) for the HPLC and phytochemical characterization of the product, and Mrs. Paola Montenegro for her skillful administrative assistance and devoted concern for all patients of this study.

Disclosures None

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