



Inhibitory effects of andrographolide on activated macrophages and adjuvant-induced arthritis

Swati Gupta¹ · Kamla Prasad Mishra¹ · Shashi Bala Singh¹ · Lilly Ganju¹

Received: 14 April 2017 / Accepted: 12 July 2017 / Published online: 22 July 2017
© Springer International Publishing AG 2017

Abstract Andrographolide, a diterpenoid lactone obtained from plant *Andrographis paniculata*, is used in South Asian countries to relieve various inflammatory symptoms. To study the effects of this agent, the impact of andrographolide on production of inflammatory mediators were delineated in mouse peritoneal macrophages (PM ϕ). Inflammatory mediators like nitric oxide (NO), tumor necrosis factor (TNF)- α , interleukin-6 and related molecular mechanisms of andrographolide-mediated inhibition of enzymes/transcription factors were studied. In addition, the in vivo anti-inflammatory activity of andrographolide was evaluated in an adjuvant-induced arthritis rat model. The results indicated that andrographolide clearly inhibited the production of NO and TNF- α in lipopolysaccharide-activated PM ϕ in a dose-related manner. Immunoblot analyses revealed that andrographolide suppressed activation of both inducible NO synthase and cyclo-oxygenase-2 by directly targeting nuclear transcription factor (NF)- κ B. Complete Freund's Adjuvant-induced paw edema in rats was also significantly inhibited by andrographolide treatment. From the data, we concluded that andrographolide imparted anti-inflammatory effects by suppressing two key inflammatory enzymes and a signaling pathway that mediates expression of variety of inflammatory cytokines/agents in situ. It is plausible that eventually, after further toxicologic characterization, andrographolide might be useful as a drug for the clinical treatment of various

inflammatory diseases like rheumatoid arthritis or diseases associated with joint pain.

Keywords Inflammation · Cytokines · Nitric oxide · Macrophages · NF- κ B

Introduction

Macrophage activation occurs rapidly after endotoxin exposure. Activated macrophages, in turn, release various pro-inflammatory cytokines and inflammatory mediators such as nitric oxide and reactive oxygen species (ROS) that have immuno-protective functions (Duque and Descoteaux 2014). However, when over-activated, macrophage-mediated inflammatory responses may exacerbate damage to tissues (Park et al. 2015). It is thus understandable that increased levels of activated macrophages are observed in various human inflammatory diseases, including colitis (Akagi et al. 2014), inflammatory bowel disease (Fries et al. 2013), arthritis (Wagner-Weiner 2015), and many other pathologies. Therefore, it is important to tightly regulate the inflammatory response generated by activated macrophages without overtly compromising the host immune system.

Over the years, a plethora of plant-derived anti-inflammatory compounds have been extracted and tested for use in treating inflammatory diseases (Wang et al. 2013; Zündorf and Fürst 2014). One compound is andrographolide (Fig. 1) extracted from the aerial parts of plant *A. paniculata* (family: *Acanthaceae*). Andrographolide, a labdane diterpenoid, is the major bioactive compound of *A. paniculata*. This agent has been shown to impart protective effects in respiratory tract infection (Poolsup et al. 2004), rheumatoid arthritis (Burgos et al. 2009), gastritis (Saranya

✉ Kamla Prasad Mishra
kmpmgi@rediffmail.com

¹ Sc. 'E' Immunomodulation Laboratory, Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi 110054, India

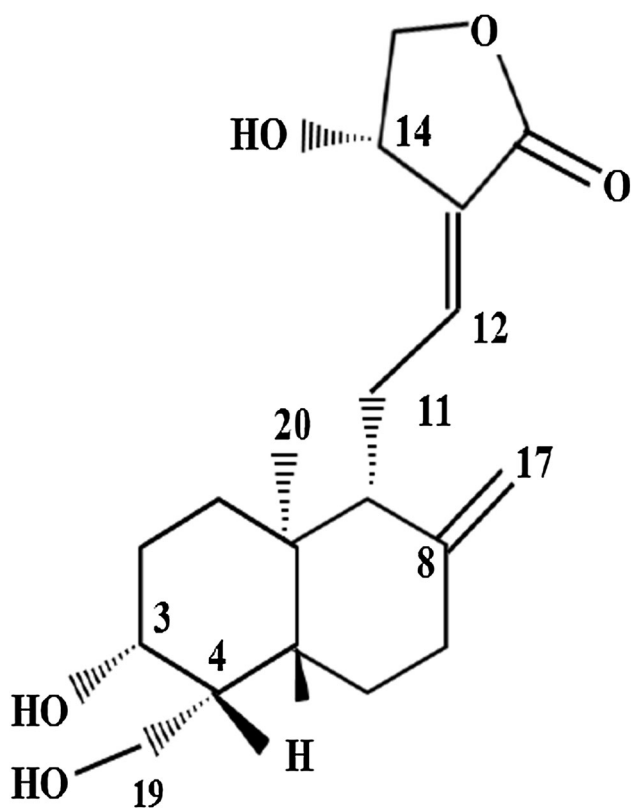


Fig. 1 Chemical structure of andrographolide [C₂₀H₃₀O₅]

and Geetha 2011), and neuropathies (Das et al. 2009), as well as impart antiviral (Gupta et al. 2016), -bacterial (Arifullah et al. 2013), and -cancer (Lim et al. 2012) effects. Similar effects had been noted when whole plant extract was tested in various pathologies (Tang et al. 2012; Suriyo et al. 2014).

LPS initiates acute inflammatory responses and enhances the *in vivo/in vitro* production of inflammatory cytokines like tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and of inflammation-associated agents/second messengers like nitric oxide (NO), and activation of enzymes like inducible nitric oxide synthase (iNOS). Among many immune-related pathologies, arthritis is associated with joint inflammation and concomitant joint destruction. An increased localized/systemic release of TNF- α and IL-1 β contributes to the joint swelling and cartilage destruction associated with the disease (Lagha et al. 2015). While there are suggestions from the earlier-cited studies that andrographolide inhibits formation of inflammatory mediators in different ailments, mechanisms underlying these outcomes are poorly understood. Accordingly, the study reported here sought to elucidate anti-inflammatory effects of andrographolide *in vitro* in LPS-activated macrophages and *in vivo* in an acute adjuvant-induced arthritis (AIA) rat model. Furthermore, to understand some of the mechanisms of action of this

compound, potential targets in inflammatory pathways were also evaluated.

Materials and methods

Preparation of andrographolide solution

Andrographolide (Sigma, St. Louis, MO) stock solution were prepared, i.e., 10 mg/ml for *in vitro* experiments and 20 mg/ml for *in vivo* experiments by dissolving in dimethyl sulphoxide (DMSO; cell culture grade, Sigma) and stored at 4 °C. Working solutions were prepared by diluting in phosphate-buffered saline (PBS, pH 7.4).

Animals

Balb/c mice (male, 25–30 g, 4-week-of-age) and Sprague–Dawley rats (male, 100–120 g, 4-week-of-age) were both obtained from the Experimental Animal Facility of Defence Institute of Physiology and Allied Sciences (DIPAS), Delhi. All animals were housed in specific-pathogen-free facilities at DIPAS that were maintained at 25 °C with a 50% relative humidity and a 12-h light:dark cycle. All protocols were approved by the DIPAS Institutional Animal Ethics Committee (IAEC/DIPAS/2015-25, 18/10/2015).

Cells

To isolate peritoneal macrophages (PM ϕ), 1 ml of 4% (w/v) starch solution (Sigma) was injected into the mouse peritoneal cavity. Two days later, animals were euthanized with ether and PM ϕ were obtained by injecting chilled PBS into the cavity (5 ml, once). After gentle massaging of the abdomen, the solution was withdrawn and then centrifuged at 300 \times g for 5 min at 4 °C. The final cell pellets were re-suspended in complete RPMI-1640 medium (RPMI supplemented with 10% fetal bovine serum (FBS), 100 U penicillin/ml, 0.1 mg streptomycin/ml, and 0.25 μ g amphotericin B/ml [all Sigma]).

Cell toxicity

PM ϕ (10⁶ cells/ml) were seeded into 96-well flat-bottom plate (200 μ l/well) and then treated with andrographolide at doses 0.5, 1.0, 5.0, or 10 μ g/ml (A0.5, A1, A5, A10) with and without LPS 1 μ g/ml (lipopolysaccharide, Type 026:B6 from *Escherichia coli*; Sigma), and incubated at 37 °C in a 5% CO₂ atmosphere. All samples were assayed in triplicate. After 48 h, 20 μ l of MTT 0.5 mg/ml solution (Sigma, USA) was added to each well and the plates were incubated further for 4 h at 37 °C. After this, the culture supernatant was removed from each well and the formazan

crystals that had formed in live cells were dissolved by the addition of 200 μ l DMSO to each well. The optical density was measured at 570 nm using a 96-well plate reader (Biotek Instruments, Winooski, VT). Relative survival of both LPS⁺ and LPS⁻ cells was calculated from comparisons of mean OD values vs. that in control wells.

Nitric oxide (NO) formation

Naïve PM ϕ in complete RPMI 1640 were plated in 96-well culture plate (at 10⁶ cells/ml; 200 μ l/well) and cultured at 37 °C in a 5% CO₂ atmosphere. After 24 h, half of the media was replenished with fresh media and andrographolide treatment at various doses was given in the presence or absence of LPS (1 μ g/ml). After incubation at 37 °C for 48 h, nitrite (as surrogate for NO) was measured from 100 μ l of supernatant with an equal volume of Griess reagent (0.2% naphthylethylenediamine dihydrochloride, and 2% sulphanilamide in 5% phosphoric acid; Sigma) at 540 nm using microplate reader (Biotek). Levels of NO in each sample were extrapolated from a standard curve that had been generated in parallel using sodium nitrite standards. All samples were assayed in triplicate.

Protein expression

To determine effects of treatment of andrographolide on expression of iNOS, COX-2, and NF- κ B, cytoplasmic and nuclear extracts of 5 \times 10⁶ naive PM ϕ were prepared in the presence of protease inhibitor cocktail (Sigma), as per standard protocols (Mishra et al. 2006). After determining the protein content using Bradford's reagent, equal amounts of protein (40 μ g) were loaded in 12% SDS gels. After resolution, the gel materials were electro transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). The PVDF membrane was then blocked for 1 h at room temperature (RT) with 3% bovine serum albumin (BSA, Sigma) in Tris-buffered saline (TBS). The membrane was then incubated overnight at 4 °C in a TBS solution containing rabbit anti-mouse COX-2 (1:200; Biorbyt, Berkeley, CA), anti-iNOS (1:1000, Biorbyt), and anti-NF- κ B (1:1000, Biovision, Milpitas, CA). After washing, membrane was incubated for 45 min at 28 °C in a TBS solution containing horseradish peroxidase-conjugated anti-rabbit IgG (1:30,000, Sigma). After a final series of washes, each membrane was processed for presence of the protein using a chemiluminescence development system. The membranes were then assessed for expression using Image J software (NIH, Bethesda, MD) and densitometric values obtained were used for comparison of expression of the proteins.

Cytokine formation

PM ϕ in complete RPMI-1640 were seeded in 96-well plate at 10⁶ cells/ml (200 μ l/well). Cells were then treated with and without LPS (1 μ g/ml) along with different doses of andrographolide (0.5–10 μ g/ml) and the plates then incubated at 37 °C for 24 h. Thereafter, supernatants from each group were harvested and stored at –80 °C until assayed. Levels of TNF- α and IL-6 were estimated using commercial ELISA kits (eBioscience, San Diego, CA), according to manufacturer instructions. All samples were assayed in triplicate. The level of sensitivity of TNF- α and IL-6 kit was 8 and 2 pg/ml, respectively.

Arthritis measures

Adjuvant-induced arthritis (AIA) was induced in Sprague–Dawley rats by injecting 100 μ l Complete Freund's Adjuvant (CFA) (Sigma, USA) intradermally on day 1 into the left footpad according to the method of de Castro Costa et al. (1981). Andrographolide treatment was then given intraperitoneally (1000 μ l/injection) at 50 mg/kg body weight on five alternate days (i.e., days 1, 3, 5, 7, and 9) (Wen et al. 2014). As a positive control, dexamethasone (DEXA) (Sigma, USA) was injected intraperitoneally (100 μ l/injection; 6 mg/kg) into a separate set of AIA rats on days 1, 3, 5, 7, and 9) (Jayashankar et al. 2012). To assess AIA status, 24 h after either andrographolide or DEXA treatment, the thickness of ossicular tissues was measured using a Gneupel caliper (Zurich, Switzerland) on days 2, 4, 6, 8, and 10—as well as on day 12.

Statistical analysis

All results were expressed as mean \pm SE. All statistical comparisons were carried out using a Students' independent *t* test and a one-way analysis of variance (ANOVA) wherever applicable. Significance was set at *p* < 0.05. All analyses were performed using SPSS-16 software (SPSS Inc, Chicago, IL).

Results

Dose dependent effects of andrographolide

Andrographolide cytotoxicity

Cytotoxic effects of andrographolide were estimated using MTT assay in mouse PM ϕ s treated with various concentrations of andrographolide (with and without LPS) for 48 h. The results showed that among LPS⁻ cells, andrographolide at 0.5, 1 and 5 μ g/ml resulted in a low albeit

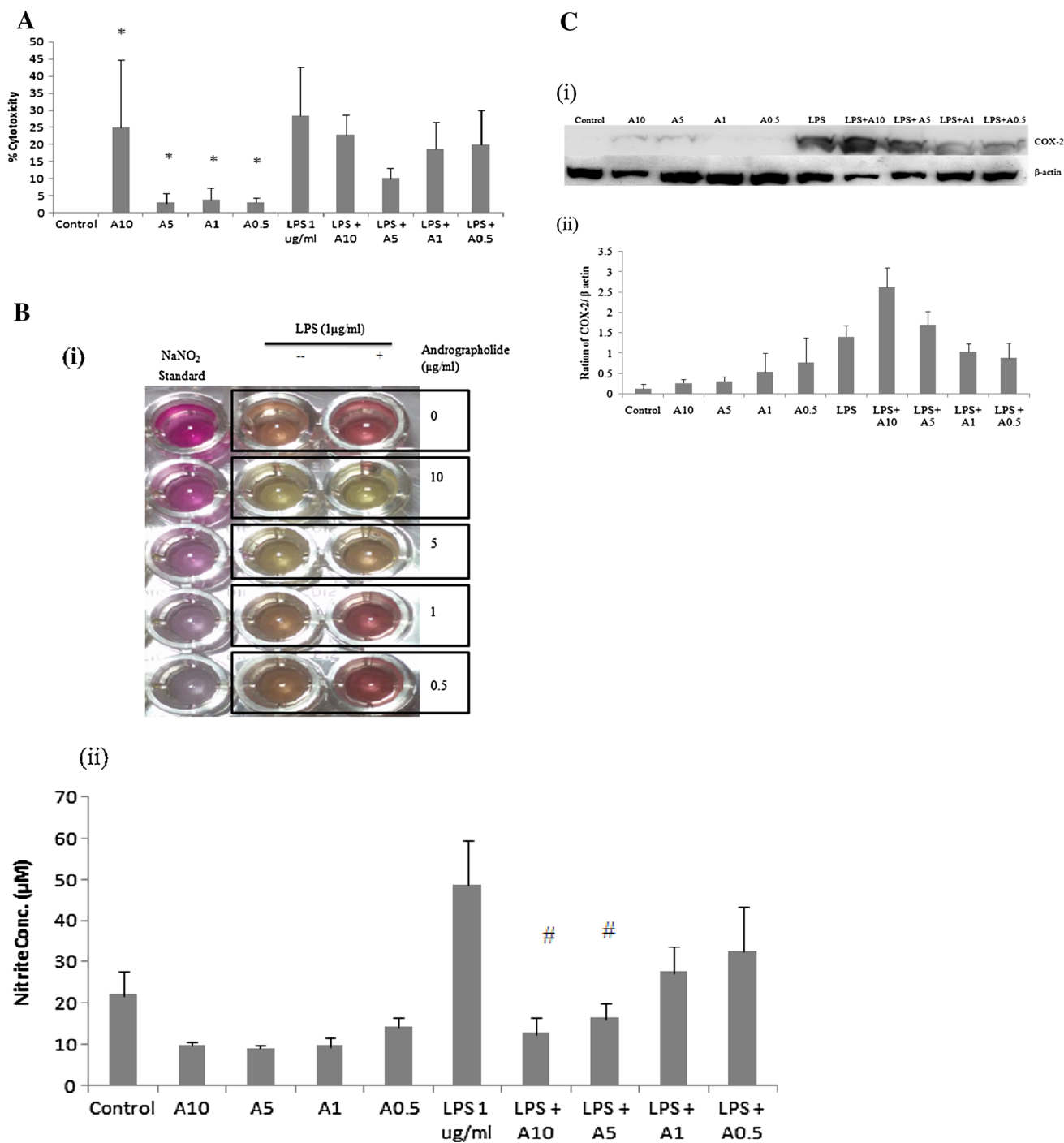


Fig. 2 Dose-dependent effect of andrographolide treatment on cell viability, NO production and COX-2 protein expression. Figure 2a represents the percent cytotoxicity and 2b represents the NO production on andrographolide treatment to mouse PM ϕ with and without LPS stimulation. Figure 2b (i) show the pictorial presentation of dose dependent color development on addition of Griess reagent. Figure 2b (ii) demonstrates quantitative release of NO. Figure 2c represents dose-dependent COX-2 protein expression. (i) presents a representative immunoblot and the bottom figure (ii) is associated

densitometric evaluation (showing ratio vs. control level) of the blot. Cells stimulated with LPS at 1 μ g/ml in the presence and absence of 0.5, 1, 5, and 10 μ g/ml andrographolide (A0.5, A1, A5, and A10) were studied. Values shown in Fig. 2a are mean \pm SE % cytotoxicity ($n = 4$ /treatment), 2b (ii) are mean \pm SE μ M NO ($n = 4$ /treatment) and 2c (ii) are mean \pm SE relative expression levels ($n = 3$ /treatment). *Value significantly different from control (naïve) cells at $p < 0.05$ and #Value significantly different from LPS only cells at $p < 0.05$

significant less than 4% toxicity as compared to untreated control cells. However, andrographolide treatment at 10 $\mu\text{g/ml}$ caused 25% cytotoxicity to the cells (Fig. 2a). Among LPS⁺ cells, the co-presence of andrographolide led to diminution of toxicity from LPS itself; however, these levels of toxicity were not significant. The data, therefore, showed that lower doses of andrographolide, i.e., 1, 5 and 0.5 were non toxic to the cells.

Effect of andrographolide on LPS induced NO production

The concentration of nitrite in cell supernatants was determined by Griess Reagent. After 48 h of treatment of the PM ϕ with LPS (1 $\mu\text{g/ml}$) alone and in combination with various doses of andrographolide, dose dependent NO production was observed (Fig. 2b (i), (ii)). It was found that LPS stimulation induced NO production (49 \pm 11 μM) relative to levels seen in un-stimulated cells (22 \pm 6 μM). This LPS-induced NO production was inhibited on andrographolide treatment at 0.5, or 1 $\mu\text{g/ml}$ with levels reduced to 33 \pm 11 and 28 \pm 6 μM , respectively, and significantly reduced at 5 and 10 $\mu\text{g/ml}$ to 16 \pm 4 and 13 \pm 4 μM , respectively. In cultures wherein LPS was not employed, presence of andrographolide led to some diminution of NO production from that released by cells that received medium only (control). Specifically, NO level for cultures receiving andrographolide 0.5, 1, 5, and 10 $\mu\text{g/ml}$ were 14 \pm 3, 10 \pm 2, 9 \pm 1, and 10 \pm 1 μM , respectively. The data, therefore, showed that andrographolide suppressed the LPS induced NO production.

Effect of andrographolide on COX-2 protein expression

A dose dependent study using different concentration of andrographolide was conducted to understand its effects on cells. Results showed that LPS alone increased the COX-2 protein expression to 1.4 \pm 0.27, whereas cells receiving andrographolide 0.5, 1, 5, and 10 $\mu\text{g/ml}$ on LPS stimulation showed dose independent decrease in COX-2 protein expression, i.e., 0.8 \pm 0.37, 1.0 \pm 0.20, 1.6 \pm 0.32, 2.61 \pm 0.49, respectively, as analyzed by densitometry of the blots. However, andrographolide alone showed dose dependent decrease in COX-2 protein expression (Fig. 2c). The decreased β actin expression may be attributed to the higher dose cytotoxicity caused by andrographolide at 10 $\mu\text{g/ml}$.

Effect of andrographolide on pro-inflammatory cytokine production

Changes in pro-inflammatory cytokine production on LPS stimulation in murine PM ϕ were evaluated. The results showed that cells treated with LPS alone up-regulated

TNF- α production to 3630 \pm 83 compared to control level of 6 \pm 3 pg/ml (Fig. 3a). Andrographolide treatment at 0.5, 1, 5, and 10 $\mu\text{g/ml}$ in combination with LPS reduced the expression of TNF- α to 2530 \pm 618, 2522 \pm 659, 2445 \pm 387, and 2178 \pm 268 pg/ml, respectively. Among these reductions, only that induced by andrographolide 10 $\mu\text{g/ml}$ was significant vs. the LPS-alone value. Interestingly, only the lowest dose of andrographolide (i.e., 0.5 $\mu\text{g/ml}$) significantly increased TNF- α formation relative to levels seen with naïve untreated control cells.

IL-6 levels were also up-regulated on stimulation with LPS, rising to 11,752 \pm 3557 pg/ml compared to control levels of 42 \pm 22 pg/ml (Fig. 3b). Treatment with andrographolide at 0.5, 1, 5, or 10 $\mu\text{g/ml}$ in combination with LPS reduced the inducible IL-6 production to 8305 \pm 97, 8112 \pm 13, 6949 \pm 1023, and 5170 \pm 2753 pg/ml, respectively. None of these reductions was significant vs. the LPS-alone value. Interestingly, again the lowest andrographolide dose (i.e., 0.5 $\mu\text{g/ml}$) induced the 'highest' formation of IL-6 relative to levels seen with naïve untreated control cells, though the changes were not significant.

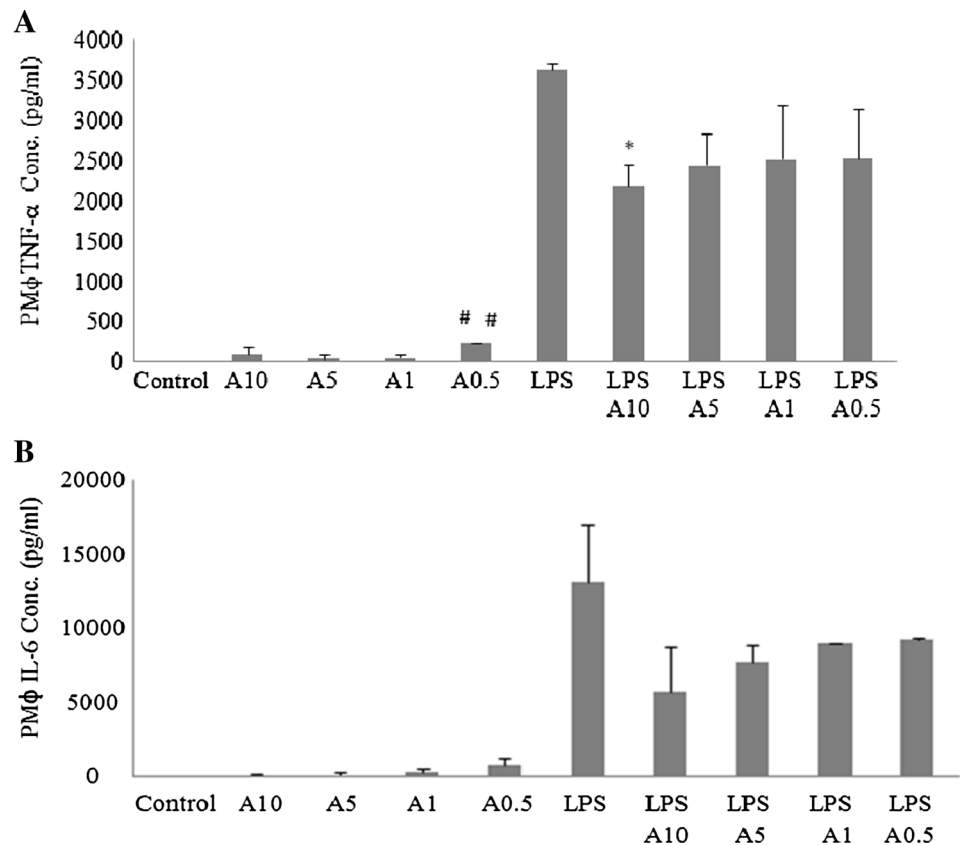
The above study showed that andrographolide at various doses has different effects. Andrographolide alone at higher concentration increases cytotoxicity, decreases -NO production, -COX-2 protein, -TNF- α and -IL-6 expressions, whereas lower doses have opposite effects. On LPS stimulation, higher doses of andrographolide showed increased COX-2 protein expression. The reason could be increased dose cytotoxicity at higher concentration of andrographolide rather than inflammation. Since increased COX-2 expression is not only associated with inflammation but also promotes cell survival by increased expression of genes involved in survival (Chang et al. 2000).

Among various doses, A0.5 showed intermediate effects, i.e., it decreased inflammation but not completely inhibited it showing its positive effect in cell recruitment at the site of infection and since the drug has decreased inflammation as compared to LPS stimulated cells, it avoids inflammation induced cell death too. Further the dose itself has least toxicity. Considering these points, we used A0.5 for COX-2, iNOS and NF- κ B protein expression.

Effect of andrographolide on LPS induced COX-2, iNOS and NF- κ B expression

The effects of andrographolide on COX-2 and iNOS protein expression in PM ϕ were evaluated using immunoblotting. The results showed that levels of each enzyme were increased on stimulation with LPS 1 $\mu\text{g/ml}$. However, treatment with andrographolide 0.5 $\mu\text{g/ml}$ to LPS induced cells decreased both COX-2 protein

Fig. 3 Dose-dependent effect of andrographolide on TNF α and IL-6 secretion. Mouse PM ϕ s were treated with 1 μ g/ml LPS in the presence and absence of various concentrations of andrographolide. Figure 3a represents TNF- α secretion and Fig. 3b represents IL-6 secretion. Values are presented as mean \pm SE pg/ml ($n = 3$ /treatment). # $p < 0.01$ vs. control, * $p < 0.05$ vs. LPS only



expression (Fig. 4a) and iNOS protein expression (Fig. 4b) relative to LPS only induced cells, though the change was not significant. Among the LPS⁻ cultures, there was no stimulatory effect on expression of either enzyme.

In Fig. 4c, LPS stimulation increased NF- κ B p65 translocation to twofold (from cytoplasm to nucleus) as compared to untreated control cells. Andrographolide treatment at 0.5 μ g/ml inhibited translocation among LPS induced PM ϕ as compared to LPS alone treated cells. While the levels of reduction were \approx 0.5-fold, however, the change was not significant. With LPS⁻ cells, andrographolide 0.5 μ g/ml reduced the translocation to 0.7-fold vs. that of naïve control cells.

Effect of andrographolide on paw edema in adjuvant induced arthritic rats

Anti-arthritic effects induced by andrographolide were noted in the CFA induced rats. On day 2, paw thickness in the CFA-only rats was \approx 8 mm compared to 2 mm in control rats. The data indicated that andrographolide (and DEXA) treatment resulted in significant reductions in paw edema from day 2 onwards (Fig. 5). At day 2, the level of reduction in paw swelling after andrographolide treatment was 24% relative to that in the CFA only induced rats (24.5% for DEXA). By day 8, there was a reduction of 27%

on andrographolide treatment relative to that of the CFA alone induced rats (32% for DEXA). Even by the final time-point, i.e., day 12, there was a 23% reduction in paw edema on andrographolide treatment relative to that of the CFA alone induced rats of same day (40% for DEXA). All changes in paw values on andrographolide treatment to CFA induced rats at each time-point were significant as compared to CFA alone rats.

Discussion

The present study reports on some potential mechanisms underlying anti-inflammatory actions of andrographolide in activated macrophages. Several lines of evidence in vivo and in vitro indicate that andrographolide is a potent anti-inflammatory agent in various pathologies (Zhang et al. 2014; Yu et al. 2015; Ren et al. 2016). Until now, these anti-inflammatory actions were attributed to inhibition of NF- κ B activation, a process involved in cytokine signaling and inflammation. However, based on the results here, it appears andrographolide may act at multiple points to impair inflammation (Fig. 6).

The effects of andrographolide on expression of COX-2 and iNOS were evaluated in LPS-activated macrophages, because andrographolide is reported to reduce translocation

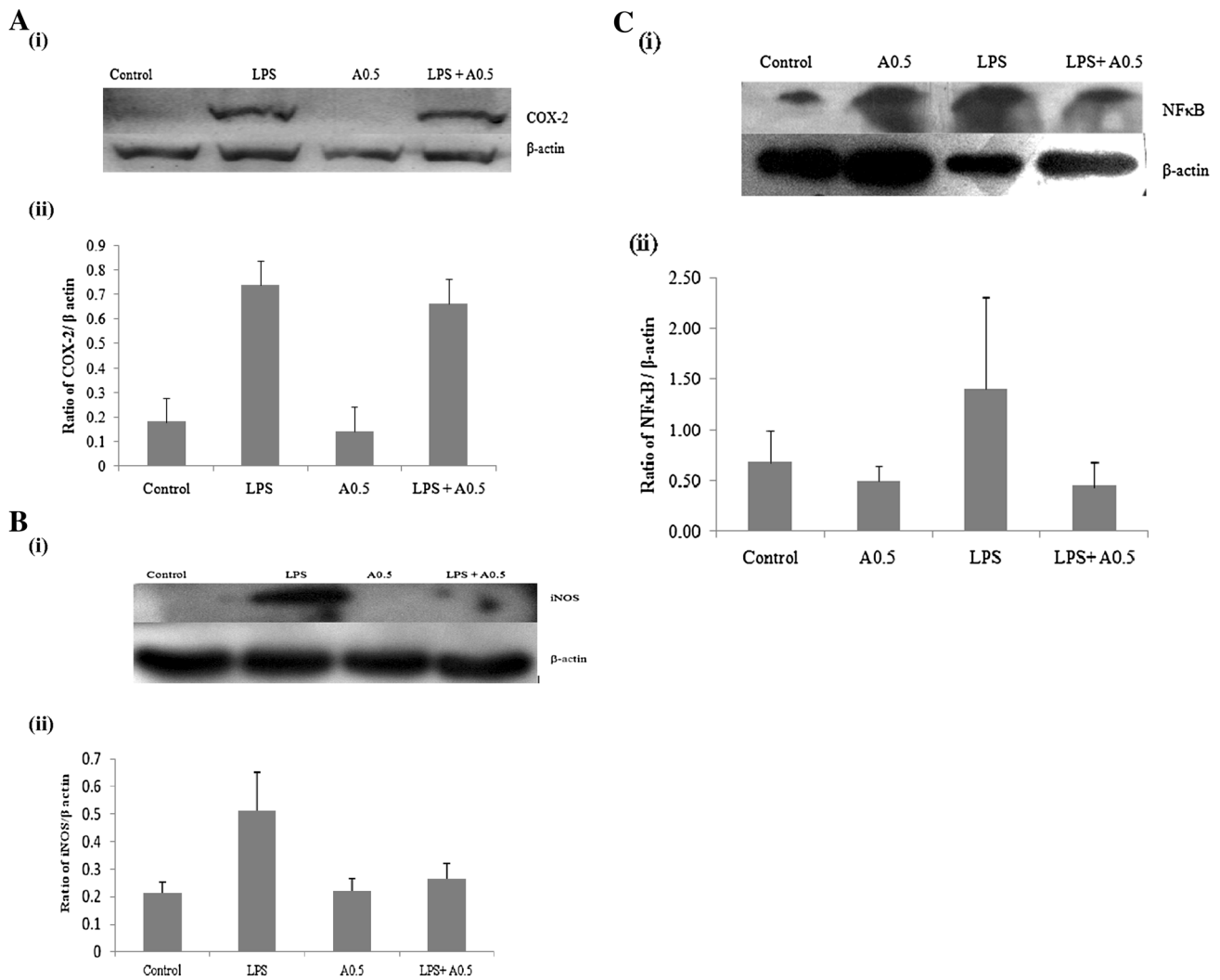


Fig. 4 Effect of andrographolide on COX2, iNOS and NF-κB protein expression. Differential expression of **a** COX-2 **b** iNOS and **c** NF-κB p65 in PMφ treated with 0.5 μg/ml andrographolide in the presence and absence of LPS (1 μg/ml). In each subfigure, the *top* portion

(*i*) presents a representative immunoblot and the *bottom* figure (*ii*) is associated densitometric evaluation (showing ratio of respective protein vs. β actin level) of the *blot*. Values shown are mean ± SE relative expression levels (*n* = 3/treatment)

of NF-κB transcription factor, an event that is associated with COX-2 and iNOS signaling (Morgan and Liu 2011). In the present study, andrographolide has shown a dose-dependent decrease in LPS induced and andrographolide treated cells as compared to LPS stimulated cells.

As expected, andrographolide suppressed the expected up-regulation of COX-2 and iNOS in activated macrophages. In general, these outcomes lent support to our hypothesis that andrographolide suppresses inflammatory responses—in part—via inhibition of inflammatory enzymes whose expression is related to expression of NF-κB or activation of NF-κB-based pathways (Hsieh et al. 2011; Chang et al. 2014). The impact on iNOS and COX-2 expression likely reflected a general effect on inflammatory cascades in activated macrophages (Zamora et al. 2000; Ricciotti and Fitzgerald 2011). In keeping with this, the

present study examined formation of inflammatory cytokines TNFα and IL-6 in stimulated cells and found that andrographolide effectively suppressed this production. It remains unclear if the effects are solely being mediated at the NF-κB-related levels as TNFα itself can activate macrophages by binding with its corresponding receptor, TNFR (Watts et al. 1999). This alternative mechanism remains to be explored and future studies using knockout mouse model will illustrate the molecules directly involved in interaction with andrographolide.

Selective pathologies that incorporate changes in iNOS and COX-2 expression, as well as in pro-inflammatory cytokine formation present excellent models to assess effects (or even mechanisms of effects) of novel pharmacologics/natural products. The CFA induced arthritic model is popularly used for the study of anti-arthritic drugs

Fig. 5 Effect of andrographolide in CFA induced acute arthritis model. Male Sprague–Dawley rats ($n = 3$) were injected with CFA in hind paw. Paw edema thickness on 6 alternate days were measured in control healthy rats (Control), CFA induced rats (CFA), CFA induced rats treated with andrographolide at a concentration of 50 mg/kg body weight (CFA + A5) and CFA induced rats treated with dexamethasone at a concentration of 6 mg/kg body weight (CFA + Dexa). * $p < 0.05$ vs. CFA. Figure 5a demonstrates pictorial presentation of paw swelling on day 6. Figure 5b demonstrates quantitative presentation of paw swelling at different days

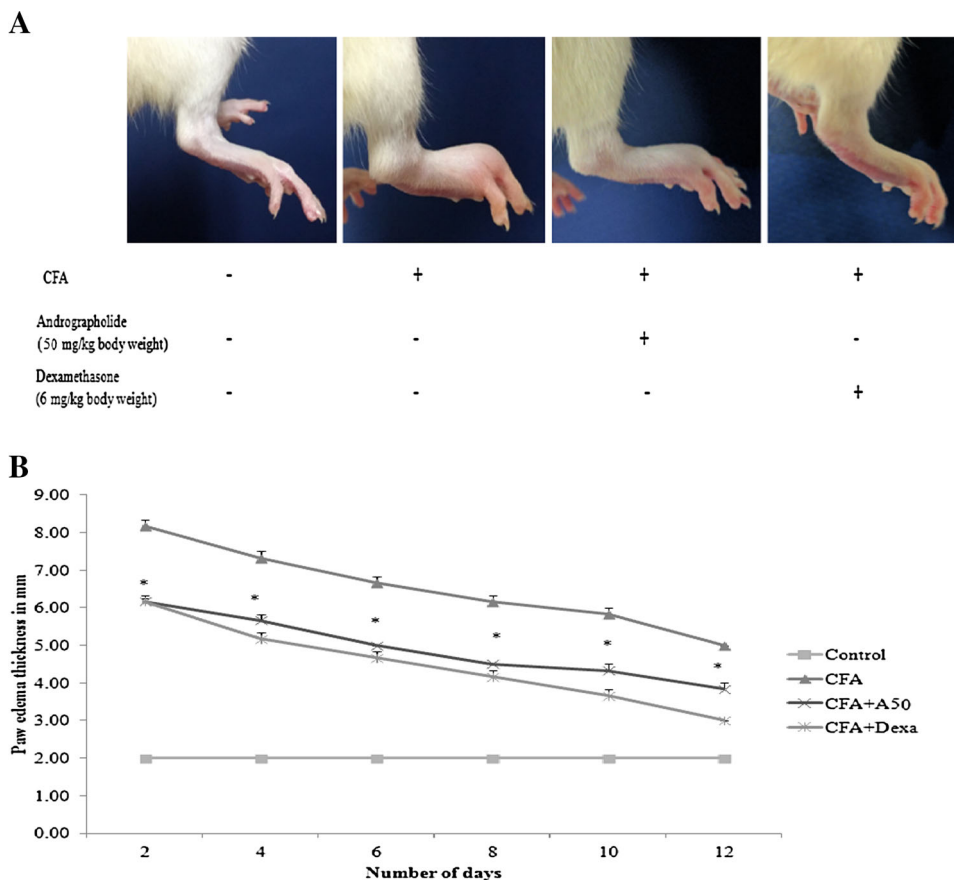
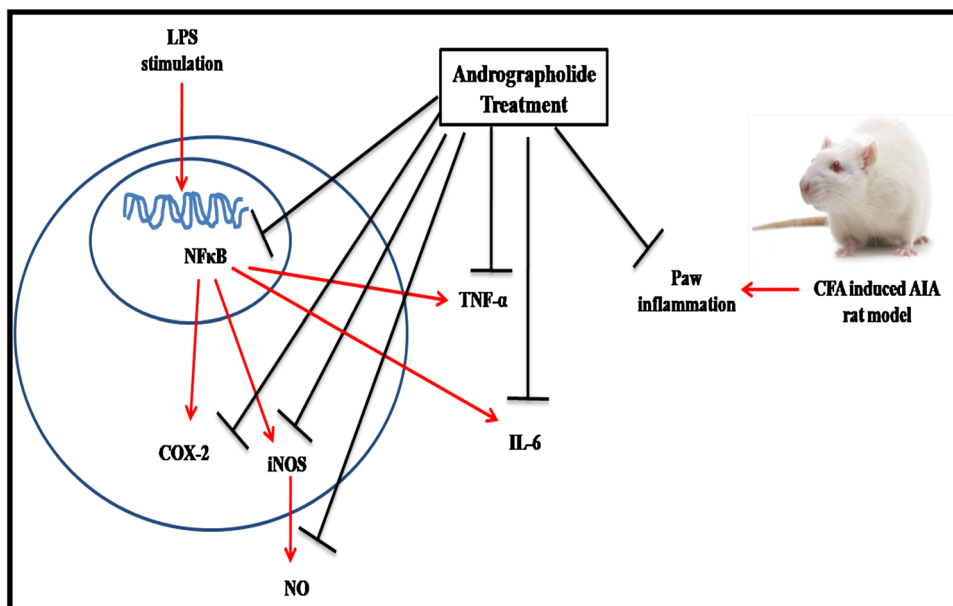


Fig. 6 Mechanisms of action of andrographolide on different inflammatory pathways



as this model shares most clinical and immunologic manifestations with human arthritis, including joint swelling, cartilage degradation and lymphocyte (i.e., T-cell) infiltration. Cytokines secreted by cells (including synovial cells, local immune cells) in the inflamed joints include IL-

17, interferon (IFN)- γ , and TNF- α also contribute to the pathology. The current study showed that administration of andrographolide produced a therapeutic effect against AIA at a level on par with that of dexamethasone, a drug renowned for its use against rheumatoid arthritis (Wang

et al. 2007). It would appear that andrographolide could become systemic and either reach the affected joints/associated cells and/or imparted effects on cells being recruited to the inflamed joints. At par effects of andrographolide to that of DEXA also proposes a question on the mechanism of action of both of these drugs in AIA model. Whether both of these drugs have same or different mechanism of action has remained unanswered. A further study is also required to check the possibility of synergistic role of andrographolide with DEXA. Nevertheless, it still remains to be determined if such anti arthritic effects of andrographolide could be translated from bench to bedside. Also, the mode of administration of andrographolide, i.e., by topical/oral dosing to an afflicted host, as the intraperitoneal route of drug administration is mostly impractical for humans.

Conclusions

This study highlighted potential pathways involved in anti-inflammatory effect of andrographolide. Proteins commonly associated with inflammatory events, i.e., NO, iNOS, COX-2, TNF- α , IL-6, and NF- κ B appeared to be impacted by andrographolide treatment in vitro. Replication of in vitro anti-inflammatory effects in in vivo would help to confirm the anti-inflammatory nature of this compound. Beyond that, confirming the use of andrographolide as an anti-inflammatory agent would help to make it plausible eventually, after further toxicologic characterization, for andrographolide to be useful as a drug for the clinical treatment of various inflammatory diseases.

Acknowledgements The Defense Research and Development Organization (DRDO) is gratefully acknowledged for financial support in the form of an NBC subproject. Author SG thanks Council of Scientific and Industrial Research (CSIR) for providing funding in the forms of research fellowship.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

References

- Akagi K et al (2014) A case of macrophage activation syndrome developing in a patient with chronic granulomatous disease-associated colitis. *J Pediatr Hematol Oncol* 36(3):e169–e172
- Arifullah M et al (2013) Evaluation of anti-bacterial and anti-oxidant potential of andrographolide and echiodinin isolated from callus culture of *Andrographis paniculata* Nees. *Asian Pac J Trop Biomed* 3(8):604–610
- Burgos RA et al (2009) Efficacy of an *Andrographis paniculata* composition for the relief of rheumatoid arthritis symptoms: a prospective randomized placebo-controlled trial. *Clin Rheumatol* 28(8):931–946
- Chang YW et al (2000) Cyclooxygenase 2 promotes cell survival by stimulation of dynein light chain expression and inhibition of neuronal nitric oxide synthase activity. *Mol Cell Biol* 20(22):8571–8579
- Chang CC et al (2014) Andrographolide, a novel NF- κ B inhibitor, inhibits vascular smooth muscle cell proliferation and cerebral endothelial cell inflammation. *Acta Cardiol Sinica* 30(4):308–315
- Das S et al (2009) Oxidative stress in the brain of nicotine-induced toxicity: protective role of *Andrographis paniculata* Nees and vitamin E. *Appl Physiol Nutr Metab* 34(2):124–135
- De Castro Costa M et al. (1981) Adjuvant-induced arthritis in rats: a possible animal model of chronic pain. *Pain* 10(2):173–185
- Duque GA, Descoteaux A (2014) Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol* 5:491–503
- Fries W, Cottone M, Cascio A (2013) Systematic review: macrophage activation syndrome in inflammatory bowel disease. *Aliment Pharmacol Ther* 37(11):1033–1045
- Gupta S, Mishra KP, Ganju L (2016) Broad-spectrum antiviral properties of andrographolide. *Arch Virol*. doi:10.1007/s00705-016-3166-3
- Hsieh CY et al (2011) Andrographolide enhances nuclear factor-kappaB subunit p65 Ser536 dephosphorylation through activation of protein phosphatase 2A in vascular smooth muscle cells. *J Biol Chem* 286(8):5942–5955
- Jayashankar B et al (2012) A supercritical CO₂ extract from seabuckthorn leaves inhibits pro-inflammatory mediators via inhibition of mitogen activated protein kinase p38 and transcription factor nuclear factor- κ B. *Int Immunopharmacol* 13(4):461–467
- Lagha A et al (2015) Interleukin-1 β , Interleukin1-Ra, Interleukin-10, and tumor necrosis factor- α polymorphisms in Tunisian patients with rheumatoid arthritis. *Pathol Biol* 63(4–5):179–184
- Lim JCW et al (2012) Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. *Clin Exp Pharmacol Physiol* 39(3):300–310
- Mishra KP et al (2006) Aqueous extract of *Rhodiola imbricata* rhizome stimulates proinflammatory mediators via phosphorylated I κ B and transcription factor nuclear factor-kappaB. *Immunopharmacol Immunotoxicol* 28(2):201–212
- Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res* 21(1):103–115
- Park JH et al (2015) Macrophage activation syndrome in a newborn infant born to a mother with autoimmune disease. *J Perinatol* 35(2):158–160. doi:10.1038/jp.2014.207
- Poolsup N et al (2004) *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. *J Clin Pharm Ther* 29(1):37–45
- Ren J et al (2016) Andrographolide ameliorates abdominal aortic aneurysm progression by inhibiting inflammatory cell infiltration through downregulation of cytokine and integrin expression. *J Pharmacol Exp Ther* 356(1):137–147
- Ricciotti E, Fitzgerald GA (2011) Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol* 31(5):986–1000
- Saranya P, Geetha A (2011) Antiulcer activity of *Andrographis paniculata* (Burm.F.) Wall. against cysteamine-induced duodenal ulcer in rats. *Indian J Exp Biol* 49(7):525–533
- Suriyo T et al (2014) *Andrographis paniculata* extracts and major constituent diterpenoids inhibit growth of intrahepatic cholangiocarcinoma cells by inducing cell cycle arrest and apoptosis. *Planta Medica* 80(7):533–543
- Tang LI et al (2012) Screening of anti-dengue activity in methanolic extracts of medicinal plants. *BMC Complement Altern Med* 12(1):3

- Wagner-Weiner L (2015) Systemic juvenile idiopathic arthritis complicated by macrophage activation syndrome. *Pediatr Ann* 44(6):e142–e147
- Wang D et al (2007) Novel dexamethasone-HPMA copolymer conjugate and its potential application in treatment of rheumatoid arthritis. *Arthr Res Ther* 9(1):R2
- Wang Q et al (2013) Naturally derived anti-inflammatory compounds from Chinese medicinal plants. *J Ethnopharmacol* 146(1):9–39
- Watts AD et al (1999) Soluble TNF-alpha receptors bind and neutralize over-expressed transmembrane TNF-alpha on macrophages, but do not inhibit its processing. *J Leukoc Biology* 66:1005–1013
- Wen L et al (2014) Activity of antibacterial, antiviral, anti-inflammatory in compounds andrographolide salt. *Eur J Pharmacol* 740:421–427
- Yu Z et al (2015) Andrographolide ameliorates diabetic retinopathy by inhibiting retinal angiogenesis and inflammation. *Biochim et Biophys Acta* 1850(4):824–831
- Zamora R, Vodovotz Y, Billiar TR (2000) Inducible nitric oxide synthase and inflammatory diseases. *Mol Med* 6(5):347–373
- Zhang QQ et al (2014) Andrographolide inhibits melanoma tumor growth by inactivating the TLR4/NF- κ B signaling pathway. *Melanoma Res* 24(6):545–555
- Zündorf I, Fürst R (2014) Plant-derived anti-inflammatory compounds: Hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. *Mediat Inflamm* 2014:1–9