

RESEARCH ARTICLE

Open Access



# The anti-inflammatory effect of *Andrographis paniculata* (Burm. f.) Nees on pelvic inflammatory disease in rats through down-regulation of the NF- $\kappa$ B pathway

Wei Zou<sup>1,2†</sup>, Zuoqi Xiao<sup>1†</sup>, Xiaoke Wen<sup>1\*</sup>, Jieying Luo<sup>3</sup>, Shuqiong Chen<sup>1</sup>, Zeneng Cheng<sup>2</sup>, Daxiong Xiang<sup>4</sup>, Jian Hu<sup>1</sup> and Jingyu He<sup>5\*</sup>

## Abstract

**Background:** *Andrographis paniculata* (Burm. f.) Nees (APN), a principal constituent of a famous traditional Chinese medicine Fukeqianjin tablet which is used for the treatment of pelvic inflammatory disease (PID), has been reported to have anti-inflammatory effect in vitro. However, whether it has pharmacological effect on PID in vivo is unclear. Therefore, the aim of this study is to test the anti-inflammatory effect of APN and illuminate a potential mechanism.

**Methods:** Thirty-six female specific pathogen-free SD rats were randomly divided into control group, PID group, APN1 group, APN2 group, APN3 group and prednisone group. Pathogen-induced PID rats were constructed. The APN1, APN2 and APN3 group rats were orally administrated with APN extract at different levels. The prednisone group rats were administrated with prednisone. Eight days after the first infection, the histological examination of upper genital tract was carried out, and enzyme-linked immunosorbent assay (ELISA) was carried out using homogenate of the uterus and fallopian tube. Furthermore, immunohistochemical evaluations of NF- $\kappa$ B p65 and I $\kappa$ B- $\alpha$  in uterus was conducted.

**Results:** APN obviously suppressed the infiltrations of neutrophils and lymphocytes, and it could significantly reduce the excessive production of cytokines and chemokines including IL-1 $\beta$ , IL-6, CXCL-1, MCP-1 and RANTES in a dose-dependent manner. Furthermore, APN could block the pathogen-induced activation of NF- $\kappa$ B pathway.

**Conclusion:** APN showed potent anti-inflammatory effect on pathogen-induced PID in rats, with a potential mechanism of inhibiting the NF- $\kappa$ B signal pathway.

**Keywords:** *Andrographis paniculata* (Burm. f.) Nees, Anti-inflammatory, Pelvic inflammatory disease, NF- $\kappa$ B, Rats

## Background

Pelvic inflammatory disease (PID) is a common gynecological disease that usually causes ectopic pregnancy, tubal factor infertility and chronic pelvic pain, which has been deemed as a great threat for life quality of woman. It includes endometritis, salpingitis, peritonitis, etc., whose

etiopathogenesis is the infection of pathogenic microorganisms in upper genital tract [1]. Infiltrations of neutrophils and lymphocytes in the upper genital tract could be employed as a criteria to diagnose PID in clinic, which could be observed on hematoxylin and eosin (H & E)-stained biopsy sections [2]. With the inflammatory cell recruitment, amount of proinflammatory cytokines, such as interleukin (IL)-1 $\beta$ , IL-6, etc., are released excessively in local tissue and play an important role in the pathogenesis of PID [3]. The important reason for the excessive production and release of proinflammatory cytokines is the activation of nuclear factor-kappa

\* Correspondence: wxk19631214@sina.com; jy.he@giat.ac.cn

†Equal contributors

<sup>1</sup>Key Laboratory of Hunan Province for Traditional Chinese Medicine in Obstetrics and Gynecology Research, Hunan Provincial Maternal and Child Health Care Hospital, No. 53 XiangChun Road, Changsha 410008, China

<sup>5</sup>Guangzhou Institute of Advanced Technology, Chinese Academy of Sciences, No. 1121 Haibin Road, Guangzhou 511458, Guangdong, China

Full list of author information is available at the end of the article



B (NF- $\kappa$ B) signaling pathway when pathogens are recognized by their receptors [4].

According to the guidelines from the Center for Disease Control and Prevention (CDC) in US, antibiotics is the first choice for the treatment of PID [5], but the bacterial drug resistance and drug side effects are shortages for clinical use of antibiotics. Therefore, new complementary medicines used for PID are needed for further improvement in clinical outcomes. *Andrographis paniculata* (Burm. f.) Nees (APN) is a famous traditional medicine, widely used to treat sore throat, flu, and upper respiratory tract infections in many Asian countries [6]. Phytochemical studies on APN have found the principal bioactive compound andrographolide [7] and many other constituents, including diterpenoids, flavonoids, quinic acids, xanthenes [8, 9], and noriridoids [10, 11]. Based on the abundant bioactive constituents, APN showed many salutary effects, such as anticancer [12], hepatoprotective [13], antiviral [14, 15], antipyretic and analgesic [16] effects, *etc.* Interestingly, the immunomodulatory [17], antioxidant [18], anti-inflammatory [19] and antimicrobial [20, 21] activities of APN were also reported, which may suggest the clinical use of APN in treatment for PID. Besides, APN is the principal constituent in a famous traditional Chinese medicine Fukeqianjin tablet which is used to treat PID. However, whether APN has pharmacological effect on PID has not been eliminated yet. In this study, we test the anti-inflammatory effect of APN on PID rats, and illustrate a potential mechanism of this activity.

## Methods

### Reagents and materials

Pentobarbital was from Xiya Reagent (Chengdu, China). Progesterone injection was obtained from Xianju Pharma (Taizhou, China). Distilled water was used in all of this experiment. Absorbable gelatin sponge was from Jinling Pharmaceutical Co. (Nanjing, China). The dried APN was purchased from Tianxiang Co. (Yueyang, China), which was identified by Prof. Zhuxin Wang (Hunan university of Chinese medicine, Changsha, China), with the total amount of andrographolide and dehydroandrographolide about 1%. A voucher specimen (No. CXL20150610) is deposited in the Key Laboratory of Hunan Province for Traditional Chinese Medicine in Obstetrics & Gynecology Research (Changsha, China). Prednisone acetate tablets were obtained from Guangdong Huanan Pharma (Guangdong, China). According to the previous method [22], a 1000 g of dried APN was extracted with 80% ethanol at room temperature and filtrated. The solution was freeze-dried to yield 62.5 g of the APN extract.

### Rat PID model construction and sample collection

The animal experimental procedure was approved by the Animal Care and Use Committee of Central South

University. Thirty-six female specific pathogen-free SD rats, 9-week aged and weighing 220–240 g, were randomly divided into 6 groups, including control group, PID group, APN1 group, APN2 group, APN3 group and prednisone group. Rats were acclimated for 7 days and then injected subcutaneously with 10 mg progesterone. One week later, the PID model construction was carried out referring to our previous method with some revisions [23]. Absorbable gelatin sponge, a volume of 0.125 ml, was immersed in microbe-mixing solution with *U. urealyticum* (t-strain mycoplasma) concentration of  $1 \times 10^8$  ccu/ml and pathogenic *E. coli* concentration of  $1 \times 10^8$  cfu/ml. Each upper genital tract of all rats except control group rats was inserted with a microbe-containing gelatin sponge, and then the rat was forced to be down for 3 min. The microbe-free gelatin sponges were implanted into the cervixes of control group rats. Four times infections were conducted with a 2-day interval. From the first infection, the APN1, APN2 and APN3 group rats were orally administrated with APN extract at a dose of 167, 334 (clinical dose) and 668 mg/kg/day, respectively. The prednisone group rats were administrated with prednisone at a dose of 1.7 mg/kg. Eight days after the first infection, rats were injected subcutaneously with pentobarbital at a dose of 30 mg/kg. The right uterus and fallopian tube were collected and restored at  $-80^\circ\text{C}$ , and the left uterus and fallopian tube was immersed in neutral-buffered formalin (10%). At last, rats were sacrificed by cervical dislocation.

### Histological evaluation

After paraffin embedding, the left uterus and fallopian tube was cut into 2  $\mu\text{m}$  sections, followed by staining with H & E. The semi-quantification was carried out according to the previous method [24]. Three parts of each slide (tissue) were checked under microscopy ( $\times 100$ ) by a blinded observer. The inflammation of each uterus and fallopian tube was semi-scored by evaluation on the extent of inflammatory cells infiltration (graded from 0 to 3).

### Enzyme-linked immunosorbent assay (ELISA)

Each right uterus and fallopian tube was weighted, immersed in physiologic saline at the ratio of 5:1 (v/w), and homogenized. The amounts of IL-1 $\beta$ , IL-6, CXCL-1, MCP-1 and RANTES in homogenate were determined by using ELISA kits (Neobioscience, Beijing, China). The total protein in tissue homogenate was measured with bicinchoninic acid (BCA) protein assay kit (Beyotime, Shanghai, China), and concentrations of these cytokines and chemokines were expressed as  $\mu\text{g/g}$  protein of homogenate.

### Immunohistochemical evaluation

The paraffin embedded uterus was cut in to 2  $\mu\text{m}$  sections, and then the paraffin was removed through xylene. The slide was boiled in 10 mM sodium citrate (pH 6.0) for

30 min for antigen retrieval. Non-specific binding sites were blocked by bovine serum. The specimens were incubated with NF- $\kappa$ B p65 or I $\kappa$ B- $\alpha$  primary antibody (Abclonal, Maryland, US) at 37 °C followed by secondary antibodies incubation. After that, the slices were treated with diaminobenzidine (DAB) reagent for color development, followed by hematoxylin counterstaining. For negative control, phosphate-buffered saline (PBS) was used instead of primary antibodies. The slices were observed under light microscopy at a magnification of  $\times$  400. Brown staining in uterus was considered as NF- $\kappa$ B p65 or I $\kappa$ B- $\alpha$ . The nuclear with translocation of NF- $\kappa$ B p65 was considered as positive. The semi-quantification of I $\kappa$ B- $\alpha$  was conducted according to a previous method with some revisions [25]. The integrated optical density (IOD) values of each slide (tissue) was obtained from its three fields with the software Image-Pro Plus 6.0. IOD index of each sample was calculated as its IOD value divided by the average IOD value of control group.

#### Statistic analysis

All data are expressed as means  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) followed by Dunnett's *t* test was employed for statistical analysis. Statistical analysis was conducted by using SPSS 16.0. Difference was considered as statistically significant when  $p < 0.05$ .

## Results

### APN attenuated the infiltration of inflammatory cells

The results of histological evaluation are showed in Fig. 1. Mass inflammatory cells, including neutrophils and lymphocytes, infiltrated into the uterus and fallopian tube of the PID group rat, indicating the occurrence of inflammation in upper genital tract. After administration of APN, the infected rats showed decreased infiltrations of neutrophils and lymphocytes, compared to PID group rats. Therefore, APN could suppress the infiltration of inflammatory cells in the upper genital tract of PID rats.

### APN reduced the excessive production of cytokines and chemokines

To observe the inflammatory response and investigate the reason of inflammatory cells infiltration in the uterus and fallopian tube, the IL-1 $\beta$ , IL-6, CXCL-1, MCP-1, and RANTES in tissue homogenate were determined by using ELISA kits. As showed in Fig. 2 all these cytokines or chemokines were excessively produced in PID group compared to control group, and APN significantly reduced this pathogens-induced excessive production in a dose-dependent manner.

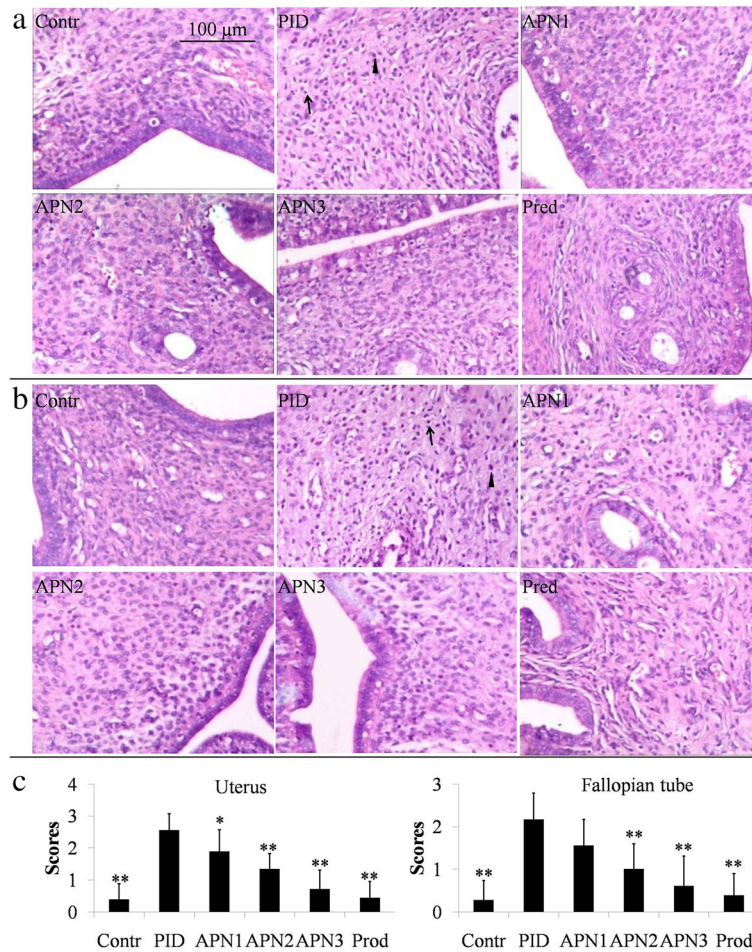
### APN exerted its anti-inflammatory effect via down-regulating the NF- $\kappa$ B pathway

To illustrate a potential mechanism of anti-inflammatory effect of APN, NF- $\kappa$ B p65 and I $\kappa$ B- $\alpha$ , two important signaling molecules in NF- $\kappa$ B signaling pathway, were determined by immunohistochemical method (presented in Figs. 3 and 4, respectively). In PID group, NF- $\kappa$ B p65 was translocated to cell nucleus, and I $\kappa$ B- $\alpha$  showed a lower content than that in the other groups. In control, APN and prednisone groups, more NF- $\kappa$ B p65 was distributed in cytoplasm. These results indicated that the NF- $\kappa$ B pathway was up-regulated in PID group, and that APN could suppress the up-regulation of signaling pathway when the upper genital tract was infected with pathogen.

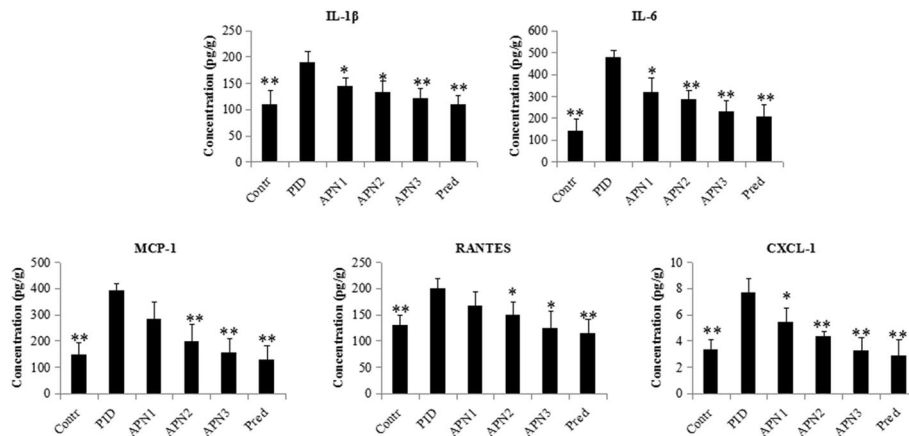
## Discussion

In clinic, the common pathogens, whose infections in upper genital tract can lead to PID, include *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, genital mycoplasmas, some gram-negative and gram-positive bacteria, etc. [26–29]. Each pathogen could be recognized by one or more members of Toll-like receptor (TLR) family and then initiate inflammation. TLR2 and TLR4 are two important members of TLR family in upper genital tract [30], and *U. urealyticum* and *E. coli* can be recognized by TLR2 and TLR4, respectively [31, 32]. Therefore, we attempted to use *U. urealyticum* and *E. coli* mixed solution to provoke an augmented inflammation in upper genital tract.

Neutrophils in blood circulation could firmly adhere to the endothelium cell barrier, cross it, and are recruited into sites of inflammation in different pathogen-infected tissues, which is the first line of innate immune defense against pathogens [33]. Recently, study also showed the infiltration of neutrophils into the endometrium of mice with lipopolysaccharides (LPS)-induced endometritis [34]. When mass neutrophils reach the tissue at the site of infection, they will release abundant inflammatory factors, oxygen free radical and proteolytic enzyme to kill pathogens. However, the excessive products will also cause tissue damage and lead to structural disease in upper genital tract. Infiltration of lymphocyte in genital tract of mice were observed after chronic pathogen genital infection, and this infiltration play a significant role in controlling the infection [35]. Additionally, researchers found more T lymphocyte and fewer plasma cells throughout the stroma and within the epithelium [36]. Unfortunately, Patton et al. reported that the tissue damage including epithelial cell degeneration occurred close approximation to lymphocytes [37]. In the present study, after chronic pathogen infections, large number of neutrophils and lymphocytes infiltrated into the epithelium of upper genital tract, and APN showed a good activity in attenuating the infiltration of these

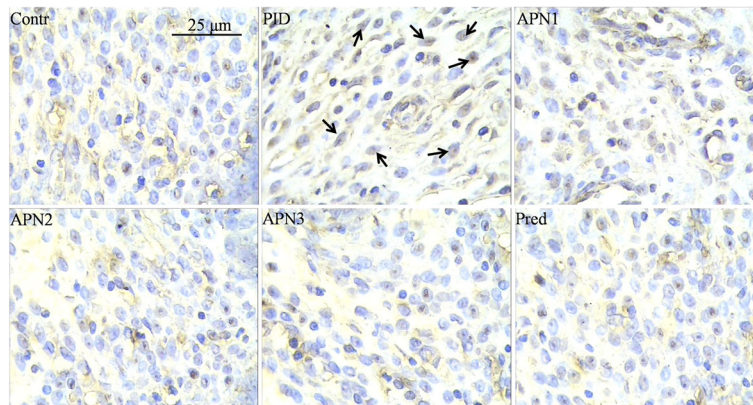


**Fig. 1** Effect of APN on pathogen-induced infiltration of neutrophils and lymphocytes in uterus and fallopian tube. Representative micrographs of uterus **a** and fallopian tube **b** stained with H & E are showed at a magnification of  $\times 100$ . The infiltration of neutrophil is indicated as  $\blacktriangle$ , and the infiltration of lymphocyte is indicated as  $\uparrow$ . **c** Histological semi-quantitative scores of inflammatory cell. Each bar represents the mean  $\pm$  SD ( $*P < 0.05$ ,  $**P < 0.01$ , significantly different from PID group;  $n = 6$ ). Contr and Pred represent control group and prednisone group, respectively



**Fig. 2** Effect of APN on pathogen-induced elevated production of IL-1 $\beta$ , IL-6, CXCL-1, MCP-1 and RANTES in uterus and fallopian tube. Each bar represents the mean  $\pm$  SD ( $*P < 0.05$ ,  $**P < 0.01$ , significantly different from PID group;  $n = 6$ )



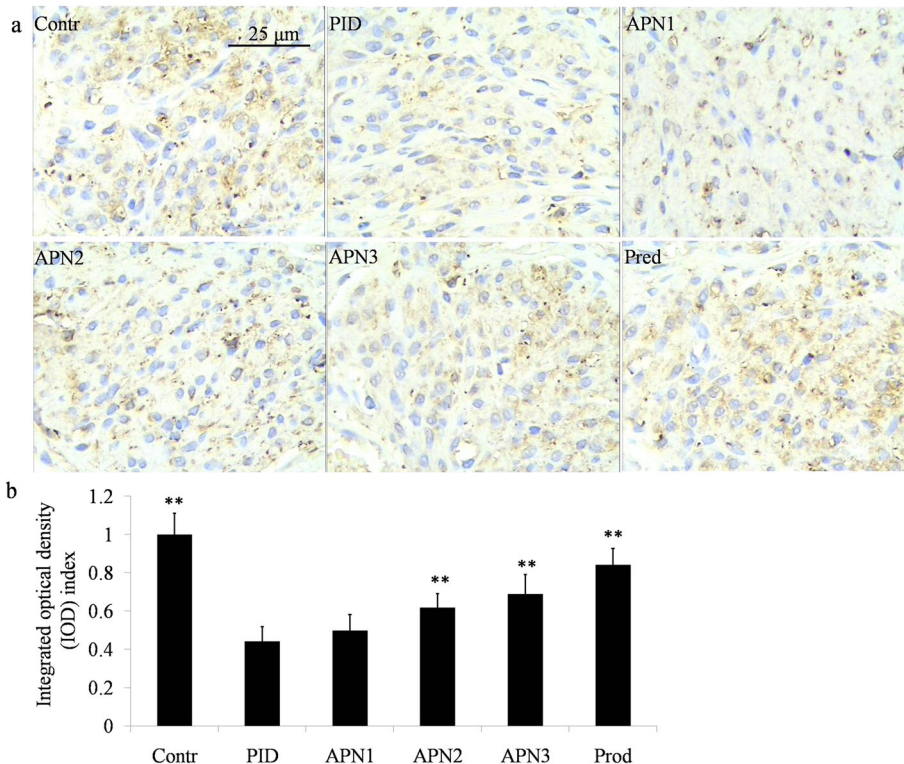


**Fig. 3** Effect of APN on pathogen-induced translocation of NF-κB p65. Representative immunohistochemistry micrographs of uterus are showed at a magnification of  $\times 400$ . Arrows indicate the representative cells with nuclear translocation of NF-κB p65 (positive nuclear)

inflammatory cells, avoiding tissue damages which is due to the inflammation.

The proinflammatory cytokines, such as IL-1 $\beta$  and IL-6, play essential roles on the initiation and propagation of inflammatory response, whose level in upper genital tract were increased with the pathogen infection and the recognition of immunogens by local TLRs [38–40]. At the site of inflammation, these proinflammatory cytokines stimulate

the proliferation and activation of leukocyte, and enhance the production of chemokines (i.e., CXCL-1, MCP-1, and RANTES) leading to the recruitment of hematopoietic immune cells. Then, activated neutrophils will release inflammatory cytokines and chemokines, and further intensify the inflammatory response [41]. At the same time, various proinflammatory cytokines will enhance the survival or function of neutrophil [42]. In this case, the tissue damage and



**Fig. 4** Effect of APN on pathogen-induced degradation of IκB-α. Representative immunohistochemistry micrographs of uterus **a** are showed at a magnification of  $\times 400$ . **b** Semi-quantitative scores of IκB-α. Each bar represents the mean  $\pm$  SD (\*\* $P < 0.01$ , significantly different from PID group;  $n = 6$ )

structural disease in upper genital tract may occur due to the intensified inflammatory response. In the present study, elevated productions of cytokines and chemokines including IL-1 $\beta$ , IL-6, CXCL-1, MCP-1 and RANTES were observed in upper genital tract of PID rats, indicating an obvious local inflammatory response. Besides, APN could significantly lower the levels of these cytokines and chemokines in a dose-dependent manner, suggesting a potent anti-inflammatory effect of APN for PID. As andrographolide is the major bioactive substance and has a critical effect on inhibiting the release of an important proinflammatory factor TNF- $\alpha$  in APN [43], it may also contribute importantly to suppressing the inflammatory response in this study.

NF- $\kappa$ B is a pivotal factor in promoting the transcription of genes involved in inflammatory and immune responses [44]. In most resting cells, NF- $\kappa$ B family members are covalently bound to I $\kappa$ B family members and located in the cytoplasm with no activity [45]. When the TLRs on surface of these cells recognize pathogens, resulting in the phosphorylation and degradation of I $\kappa$ B members, the NF- $\kappa$ B members will translocate to the nucleus and bind to the cis-acting NF- $\kappa$ B enhancer element of genes, promoting the expression of inflammatory mediators, such as IL-1 $\beta$ , IL-6, etc. [31]. These produced proinflammatory cytokines act as a positive autocrine feedback so as to the further activation of NF- $\kappa$ B, and subsequently more proinflammatory mediators are produced [46]. NF- $\kappa$ B p65 and I $\kappa$ B- $\alpha$  are representative members of NF- $\kappa$ B and I $\kappa$ B family in uterus and fallopian tube, respectively [47], and therefore were chosen as indexes to test whether the NF- $\kappa$ B pathway was activated in the present study. Our results demonstrated the activation of NF- $\kappa$ B signal pathway after multi-infection of pathogens in uterus. In the rat PID model, APN exerted its anti-inflammatory with a potential mechanism of blocking the activation of NF- $\kappa$ B pathway. Previous studies reporting that both of APN and its main substance andrographolide showed the effect on inhibiting NF- $\kappa$ B pathway in vitro support this result [48, 49]. On the other hand, andrographolide also showed the other anti-inflammatory effects, such as inhibiting JAK/STAT signaling [49], inhibiting p38 MAPK pathway [50], suppressing TLRs family expressions [49], etc., and whether APN have the same or more effects in upper genital tract should be verified in the further studies to facilitate its clinical use.

## Conclusions

In this study, oral administration of APN showed significant anti-inflammatory activity in pathogen-induced PID rats, including suppressing the infiltration of neutrophils and lymphocytes and reducing excessive production of cytokines or chemokines. A potential mechanism of this effect was involved in inhibiting the activation of NF- $\kappa$ B pathway.

## Abbreviations

APN: *Andrographis paniculata* (Burm. f.) Nees; BCA: Bicinchoninic acid; CDC: Center for disease control and prevention; DAB: Diaminobenzidine; ELISA: Enzyme-linked immunosorbent assay; H & E: Hematoxylin and eosin; IL: Interleukin; IOD: Integrated optical density; LPS: Lipopolysaccharides; NF- $\kappa$ B: Nuclear factor-kappa B; PBS: Phosphate-buffered saline; PID: Pelvic inflammatory disease; SD: Standard deviation; TLR: Toll-like receptor

## Acknowledgements

We sincerely thank Dr. Mimi Tang, Miss Yi Zheng and Miss Hui Li for assistances in animal experiment, and thank Prof. Zhuxin Wang for botanical identification.

## Funding

This research was supported by National Natural Science Foundation (No.81501218), China Postdoctoral Science Foundation (No. 2015 M582330), and Science and Technology Project of Hunan Province (No. 2015RS4054).

## Availability of data and materials

Data are included in paper.

## Authors' contributions

JL, SC and XW carried out the design of the study. WZ, ZX, XW and JH carried out the experiments. ZC and DX performed the statistical analysis. XW and WZ wrote the manuscript and made revisions. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no conflict of interest.

## Consent for publication

Not applicable.

## Ethics approval and consent to participate

The animal experimental procedure was approved by the Animal Care and Use Committee of Central South University.

## Author details

<sup>1</sup>Key Laboratory of Hunan Province for Traditional Chinese Medicine in Obstetrics and Gynecology Research, Hunan Provincial Maternal and Child Health Care Hospital, No. 53 XiangChun Road, Changsha 410008, China. <sup>2</sup>School of Pharmaceutical Sciences, Central South University, Changsha 410011, China. <sup>3</sup>College of Pharmacy, Hunan University of Chinese Medicine, Changsha 410007, China. <sup>4</sup>Clinic Pharmacy Research Laboratory, the Second Xiangya Hospital of Central South University, Changsha 410011, China. <sup>5</sup>Guangzhou Institute of Advanced Technology, Chinese Academy of Sciences, No. 1121 Haibin Road, Guangzhou 511458, Guangdong, China.

Received: 30 March 2016 Accepted: 17 November 2016

Published online: 25 November 2016

## References

- Soper DE. Pelvic inflammatory disease. *Obstet gynecol.* 2010;116(2 Pt 1):419–28.
- Vicetti Miguel RD, Chivukula M, Krishnamurti U, Amortegui AJ, Kant JA, Sweet RL, Wiesenfeld HC, Phillips JM, Chernes TL. Limitations of the criteria used to diagnose histologic endometritis in epidemiologic pelvic inflammatory disease research. *Pathol res pract.* 2011;207(11):680–5.
- Lee SA, Tsai HT, Ou HC, Han CP, Tee YT, Chen YC, Wu MT, Chou MC, Wang PH, Yang SF. Plasma interleukin-1beta, -6, -8 and tumor necrosis factor-alpha as highly informative markers of pelvic inflammatory disease. *Clin chem lab med.* 2008;46(7):997–1003.
- Epstein FH, Barnes PJ, Karin M. Nuclear factor- $\kappa$ B: a pivotal transcription factor in chronic inflammatory diseases. *N engl j med.* 1997;336(15):1066–71.
- Control CFD. Prevention: sexually transmitted diseases treatment guidelines, 2010. *Ann emerg med.* 2011;58(1):67–8.
- Jayakumar T, Hsieh CY, Lee JJ, Sheu JR. Experimental and clinical pharmacology of *andrographis paniculata* and its major bioactive phytoconstituent andrographolide. *Evid based complement alternat med.* 2013;2013:846740.
- SHARMA M, SHARMA R. Identification, purification and quantification of andrographolide from *andrographis paniculata* (burm. f.) nees by HPTLC at different stages of life cycle of crop. *J curr chem pharm sci.* 2013;3(1):23–32.

8. Subramanian R, Asmawi MZ, Sadikun A. A bitter plant with a sweet future? a comprehensive review of an oriental medicinal plant: *andrographis paniculata*. *Phytochem rev*. 2012;11(1):39–75.
9. Zhou K-L, Chen L-X, Zhuang Y-L, Wang N-L, Yao X-S, Qiu F. Two new ent-labdane diterpenoid glycosides from the aerial parts of *andrographis paniculata*. *J asian nat prod res*. 2008;10(10):939–43.
10. Sareer Q, Ahad A, Umar S. Prophylactic and lenitive effects of *Andrographis paniculata* against common human ailments: an exhaustive and comprehensive reappraisal. *J Pharm Res Opin*. 2014;2(10):138–62.
11. Xu C, Chou G-X, Wang C-H, Wang Z-T. Rare noriridoids from the roots of *andrographis paniculata*. *Phytochemistry*. 2012;77:275–9.
12. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by *andrographis paniculata* extract and andrographolide. *Immunopharmacol immunotoxicol*. 2007;29(1):81–93.
13. Rana A, Avadhoot Y. Hepatoprotective effects of *Andrographis paniculata* against carbon tetrachloride-induced liver damage. *Arch pharm res*. 1991;14(1):93–5.
14. Chang RS, Ding L, Gai-Qing C, Qi-Choa P, Ze-Lin Z, Smith KM. Dehydroandrographolide succinic acid monoester as an inhibitor against the human immunodeficiency virus. *Exp biol med*. 1991;197(1):59–66.
15. Lin T-P, Chen S-Y, Duh P-D, Chang L-K, Liu Y-N. Inhibition of the Epstein-Barr virus lytic cycle by andrographolide. *Biol pharm bull*. 2008;31(11):2018–23.
16. Madav S, Tripathi H, Mishra S. Analgesic, antipyretic and antiulcerogenic effects of andrographolide. *Indian j pharm sci*. 1995;57(3):121.
17. Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immunostimulatory compounds from *andrographis paniculata*. *J ethnopharmacol*. 2004;92(2):291–5.
18. Verma N, Vinayak M. Antioxidant action of *andrographis paniculata* on lymphoma. *Mol biol rep*. 2008;35(4):535–40.
19. Liu J, Wang Z-T, Ji L-L, Ge B-X. Inhibitory effects of neoandrographolide on nitric oxide and prostaglandin E2 production in LPS-stimulated murine macrophage. *Mol cell biochem*. 2007;298(1–2):49–57.
20. Voravuthikunchai SP, Limsuwan S. Medicinal plant extracts as anti-*Escherichia coli* O157: H7 agents and their effects on bacterial cell aggregation. *J food protect*. 2006;69(10):2336–41.
21. Xu Y, Marshall RL, Mukkur TK. An investigation on the antimicrobial activity of *andrographis paniculata* extracts and andrographolide in vitro. *Asian j plant sci*. 2006;5:527–30.
22. Zhang XF, Tan BKH. Antihyperglycaemic and anti-oxidant properties of *andrographis paniculata* in normal and diabetic rats. *Clin exp pharmacol physiol*. 2000;27(5–6):358–63.
23. Zou W, Wen X, Zheng Y, Xiao Z, Luo J, Chen S, Wang Y, Cheng Z, Xiang D, Nie Y. Metabolomic study on the preventive effect of *patrinia scabiosaefolia* fish on multipathogen induced pelvic inflammatory disease in rats. *Evid based complement alternat med*. 2015;501:170792.
24. Luo YL, Zhang CC, Li PB, Nie YC, Wu H, Shen JG, Su WW. Naringin attenuates enhanced cough, airway hyperresponsiveness and airway inflammation in a guinea pig model of chronic bronchitis induced by cigarette smoke. *Int immunopharmacol*. 2012;13(3):301–7.
25. Jiang R, Wang H, Deng L, Hou J, Shi R, Yao M, Gao Y, Yao A, Wang X, Yu L, et al. IL-22 is related to development of human colon cancer by activation of STAT3. *BMC cancer*. 2013;13:59.
26. World Health Organization. Global incidence and prevalence of selected curable sexually transmitted infections: 2008. vol. 20. In: World Health Organization, editor. *Reproductive Health Matters*; 2012. p. 207–9.
27. Quentin R, Verdon R. Microbiologic basis of diagnosis and treatment of pelvic inflammatory disease. *J gynecol obstet biol reprod*. 2012;41(8):850–63.
28. Saini S, Gupta N, Batra G, Arora DR. Role of anaerobes in acute pelvic inflammatory disease. *Indian j med microbiol*. 2003;21(3):189–92.
29. Zhang D, Wen J, Zhou W, Wu X. Pathogenic bacteria distribution and drug resistance isolated from women with pelvic inflammatory disease. *Chinese j nosocomiol*. 2009;19(13):1747–50.
30. Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR, Guyre PM. Differential expression of toll-like receptors 2 and 4 in tissues of the human female reproductive tract. *Infect immun*. 2004;72(10):5799–806.
31. He J, You X, Zeng Y, Yu M, Zuo L, Wu Y. *Mycoplasma genitalium*-derived lipid-associated membrane proteins activate NF- $\kappa$ B through toll-like receptors 1, 2, and 6 and CD14 in a MyD88-dependent pathway. *Clin vaccine immunol*. 2009;16(12):1750–7.
32. Sheldon IM, Rycroft AN, Dogan B, Craven M, Bromfield JJ, Chandler A, Roberts MH, Price SB, Gilbert RO, Simpson KW. Specific strains of *Escherichia coli* are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. *Plos one*. 2010;5(2):e9192.
33. Rebordão M, Carneiro C, Alexandre-Pires G, Brito P, Pereira C, Nunes T, Galvão A, Leitão A, Vilela C, Ferreira-Dias G. Neutrophil extracellular traps formation by bacteria causing endometritis in the mare. *J reprod immunol*. 2014;106:41–9.
34. Lv X, Fu K, Li W, Wang Y, Wang J, Li H, Tian W, Cao R. TIAA attenuates LPS-Induced mouse endometritis by suppressing the NF- $\kappa$ B signaling pathway. *Can J Physiol Pharmacol*. 2015;93(11):967–71.
35. Rank R, Soderberg L, Barron A. Chronic chlamydial genital infection in congenitally athymic nude mice. *Infect immun*. 1985;48(3):847–9.
36. Kiviat NB, Wølner-Hanssen P, Eschenbach DA, Wasserheit JN, Paavonen JA, Bell TA, Critchlow CW, Stamm WE, Moore DE, Holmes KK. Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis. *Am j surg pathol*. 1990;14(2):167–75.
37. Patton DL, Askenazy-Elbhar M, Henry-Sucher J, Campbell LA, Cappuccio A, Tannous W, Wang SP, Kuo CC. Detection of *Chlamydia trachomatis* in fallopian tube tissue in women with postinfectious tubal infertility. *Am j obstet gynecol*. 1994;171(1):95–101.
38. Tortorella C, Piazzolla G, Matteo M, Pinto V, Tinelli R, Sabbà C, Fanelli M, Cicinelli E. Interleukin-6, interleukin-1 $\beta$ , and tumor necrosis factor  $\alpha$  in menstrual effluents as biomarkers of chronic endometritis. *Fertil steril*. 2014;101(1):242–7.
39. Cronin JG, Turner ML, Goetze L, Bryant CE, Sheldon IM. Toll-like receptor 4 and MYD88-dependent signaling mechanisms of the innate immune system are essential for the response to lipopolysaccharide by epithelial and stromal cells of the bovine endometrium. *Biol reprod*. 2012;86(2):51.
40. Turner ML, Cronin JG, Healey GD, Sheldon IM. Epithelial and stromal cells of bovine endometrium have roles in innate immunity and initiate inflammatory responses to bacterial lipopeptides in vitro via toll-like receptors TLR2, TLR1, and TLR6. *Endocrinology*. 2014;155(4):1453–65.
41. Gasperini S, Zambello R, Agostini C, Trentin L, Tassinari C, Cadrobbi P, Semenzato G, Cassatella MA. Impaired cytokine production by neutrophils isolated from patients with AIDS. *Aids*. 1998;12(4):373–9.
42. Milot E, Filep JG. Regulation of neutrophil survival/apoptosis by Mcl-1. *Scientific world journal*. 2011;1:1948–62.
43. Low M, Khoo CS, Munch G, Govindaraghavan S, Sucher NJ. An in vitro study of anti-inflammatory activity of standardised *andrographis paniculata* extracts and pure andrographolide. *BMC complement altern med*. 2015;15:18.
44. Helenius M, Hänninen M, Lehtinen SK, Salminen A. Aging-induced up-regulation of nuclear binding activities of oxidative stress responsive NF- $\kappa$ B transcription factor in mouse cardiac muscle. *J mol cell cardiol*. 1996;28(3):487–98.
45. Ghosh S, May MJ, Kopp EB. NF- $\kappa$ B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu rev immunol*. 1998;16(1):225–60.
46. Bours V, Bonizzi G, Bentires-Alj M, Bureau F, Piette J, Lekeux P, Merville M-P. NF- $\kappa$ B activation in response to toxic and therapeutical agents: role in inflammation and cancer treatment. *Toxicology*. 2000;153(1):27–38.
47. Dai L, Gu L, Di W. MiR-199a attenuates endometrial stromal cell invasiveness through suppression of the IKK $\beta$ /NF- $\kappa$ B pathway and reduced interleukin-8 expression. *Mol hum reprod*. 2012;18(3):136–45.
48. Chao WW, Kuo YH, Hsieh SL, Lin BF. Inhibitory effects of ethyl acetate extract of *andrographis paniculata* on NF- $\kappa$ B trans-activation activity and LPS-induced acute inflammation in mice. *Evid based complement alternat med*. 2011;2011:254531.
49. Parichatanond W, Suthisang 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.
50. Shao ZJ, Zheng XW, Feng T, Huang J, Chen J, Wu YY, Zhou LM, Tu WW, Li H. Andrographolide exerted its antimicrobial effects by upregulation of human beta-defensin-2 induced through p38 MAPK and NF- $\kappa$ B pathway in human lung epithelial cells. *Can j physiol pharmacol*. 2012;90(5):647–53.