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Full Length Research Paper

Effect of *Angelica sinensis* aqueous extract on uterus, ovary NF-kB/β-actin and IL-6/β-actin mRNA expression level in pelvic inflammation model rats

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Angelica sinensis **is a Chinese medicinal herb for treating gynecological and gastrointestinal disorders and also in conjunction with cancer chemotherapy. In the present study, the effects of *A. sinensis* aqueous extract on uterus, ovary NF-kB/β-actin and IL-6/β-actin mRNA expression level in Pelvic inflammation model rats were investigated. Results showed that *A. sinensis* aqueous extract could significantly decreased uterus NF-kB/β-actin and IL-6/β-actin mRNA expression level in Pelvic inflammation rats.**

Keywords: Pelvic inflammation, rat, *Angelica sinensis*, NF-kB/β-actin, IL-6/β-actin.

INTRODUCTION

Pelvic inflammatory disease (or disorder) (PID) is a generic term for inflammation of the uterus, fallopian tubes and/or ovaries as it progresses to scar formation with adhesions to nearby tissues and organs (Songer et al., 2004). This may lead to infections. PID is a vague term and can refer to viral, fungal and parasitic although most often, bacterial infections. Bacteria and other micro-organisms can find their way through the vagina and the cervix to the internal reproductive organs. A bacterial infection can cause inflammation in these organs and their surroundings. This most commonly occurs in the fallopian tubes (Paavonen, 2005; Kahn et al., 1999; Somchit et al., 2011; Zhuang et al., 2011).

The rhizome of *Angelica sinensis* (Oliv.) Diels (Umbelliferae) known as Dong-gui in Chinese herb, is one of the most important traditional Chinese herbs used as a sedative or a tonic agent (Hsu and Peacher, 1976). Its varieties of pharmacological effects include anti-oxidative, anti-inflammatory, and immunomodulatory activities (Wu et al., 1999; Liu et al., 2003, Wu et al., 2011; Alipour and Khanmohammadi, 2011; Khakpour et al, 2012). The active components of *A. sinensis* (AS) include ferulic acid and polysaccharides, the main components found in the non-aromatic fractions. The ligustilide and phthalides are found in the volatile aromatic oil.

In the present study, we investigated the effect of *A. sinensis* aqueous extract on uterus, ovary NF-kB/β-actin and IL-6/β-actin mRNA expression level in Pelvic inflammation model rats.

MATERIALS AND METHODS

Plant material and preparation of the aqueous extract of Angelica sinensis

A. sinensis were purchased from a local herb shop (Yancheng, China). The plant material was dried at ambient temperature and stored in a dry place prior to use. A 300 g of the powdered aerial parts were suspended in 500 ml distilled water, heated and boiled under reflux for 30 min. The decoction obtained was filtered, and the filtrate frozen at −70°C and then lyophilized. The average yield of the lyophylised material was approximately 23% (w:w). It was stored at ambient temperature until further use.

Pelvic inflammation model

32 female SD rats (weighing 210 to 230 g) were used in the test. 10 rats were randomly taken as the normal control, sham and the other 16 rats were established as the model rats with pelvic inflammation.

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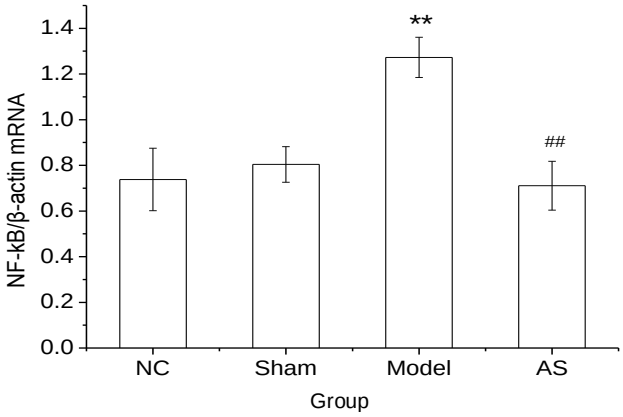

Figure 1. Effect of Angelica sinensis aqueous extract on uterus NF-kB/β-actin mRNA expression level.**P<0.01, compared with sham group; ##P<0.01, compared with model group.

The pelvic inflammation of the model rats was established by injecting 0.08 ml of 20% phenol mucilage into the right uterus of the rats to induce a pathological condition similar to pelvic inflammation. The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). 20% phenol mucilage was obtained by combining 8 ml of phenol with 32 ml of 1% carboxymethylcellulose sodium mucilage. 15 days after the model rats were established, they were randomly divided into two groups with 8 rats in each group. Sham group was injected 0.08 ml of saline into the right uterus of the rats.

The AS group received extracts of *A. sinensis* (0.4g/kg; po) for 20 consecutive days; The model control group received saline (8ml/kg. po) for 20 consecutive days; The normal control and sham group received saline (8 ml/kg. po) for 20 consecutive days.

RT-PCR

The total RNA was extracted using a ToTALLY RNA kit (Ambion Inc, Austin, TX, USA), according to the manufacturer's instructions. The NFkB sequence used was sense 5'-AGTTCAGGGGAATTTCCCAGGC-3' and the antisense 5'-GCCTGGGAAATTCCCCTGAACT-3', IL-6 sense 5'-TGGAGTTCCGTTTCTACCTG-3' and antisense 5'-TTCATATTGCCAGTTCTTCG-3'. First-strand cDNA was synthesized from 0.2 mg of total RNA using SuperScript reverse transcriptase and oligo deoxythymidine primers for PRM-2 or specific primers for NF-kB, and IL-6 mRNA with the SuperScript III First-Strand Synthesis System (Invitrogen). The cDNA synthesis was performed for 30 min at 42°C and terminated by treatment at 99°C for 5 min. Co-amplification reactions were induced in 25 ml of PCR mixtures containing 400 mM of 15-base random primers (Operon Technologies, Alameda, CA, USA), 2.5 ml 10 × Ex Taq PCR buffer (Takara Bio Inc., Shiga, Japan), 25 mM MgCl2, a mixture of dNTPs (2 mM each; Takara Bio), 2 U Ex Taq polymerase (Takara Bio), 5 ml of reverse transcribed RNA mixtures and 20 pmol each of the specific primers for NF-kB, and IL-6 mRNA. The primer sets were designed. Samples were denatured at 94°C for 3 min and sequential 35 cycles at 94°C for 1 min, 57 and 59°C (NF-kB, and IL-6 mRNA, respectively) for 1 min, and 72°C for 1 min were followed for amplification in a thermal cycler (MJ PTC-100, Global Medical Instrumentation Inc., Ramsey, MN, USA). After the last cycle, the samples were incubated for an additional 10 min at 72°C. The PCR products were separated in a 1.5% agarose gel and visualized with UV by staining with 0.05% ethidium bromide in Tris–acetate EDTA

Statistical evaluation

Significance of differences between the treated and control groups was analyzed by the Student's t test. Statistical significance was concluded at P <0.05 and 0.01.

RESULTS AND DISCUSSION

Pelvic inflammatory disease is usually the result of infection ascending from the endocervix causing endometritis, salpingitis, oophoritis, tuboovarian abscesses and/or pelvic peritonitis. Pelvic inflammatory disease is difficult to diagnose and therapeutic decisions are based on the evaluation of different parameters (clinical examination, laboratory and microbiological reports, ultrasonographic, or even laparoscopic evidence). In this study, we investigate effect of Angelica sinensis aqueous extract on uterus, ovary NF-κB/β-actin mRNA and IL-6/β-actin mRNA expression level in Pelvic inflammation model rats.

Nuclear factor kappa B (NFκB), cyclooxygenase-2 (COX-2) as well as pro-inflammatory cytokines (in particular interleukin (IL)-1β (IL-6) and tumour necrosis factor (TNF)) have been suggested to play a key role in this 5 phase mucositis model (Sonis, 2004). NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. Nuclear factor kB (NF-kB) is a nuclear transcription factor that regulates expression of a large number of genes that are critical for the regulation of apoptosis, viral replication, tumorigenesis, inflammation and various autoimmune diseases. The activation of NF-kB is thought to be part of a stress response as it is activated by a variety of stimuli that include growth factors, cytokines, lymphokines, UV, pharmacological agents and stress (Gilmore, 2006; Brasier, 2006; Perkins, 2007; Gilmore, 1999; Tian and Brasier, 2003; Kargar et al., 2011; Cervantes-Flores et al., 2011; Kolar et al., 2011). In its inactive form, NF-kB is sequestered in the cytoplasm, bound by members of the IkB family of inhibitor proteins, which include IkBa, IkBb, IkBg, and IkBe. The various stimuli that activate NF-kB cause phosphorylation of IkB, which is followed by its ubiquitination and subsequent degradation. NF-κB plays a key role in regulating the immune response to infection (kappa light chains are critical components of immunoglobulins). Incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. NF-κB has also been implicated in processes of synaptic plasticity and memory (Albensi and Mattson, 2000; Meffert et al., 2003; Levenson et al., 2004; Freudenthal et al., 1998; Merlo et al., 2002).

The results are shown in Figure 1. There was no significant difference in uterus NF-kB/β-actin mRNA

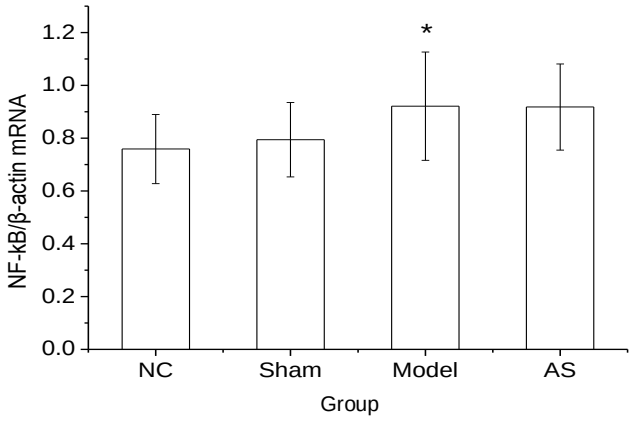

Figure 2. Effect of Angelica sinensis aqueous extract on ovary NF-kB/β-actin mRNA expression level. **P*<0.05, compared with sham group.

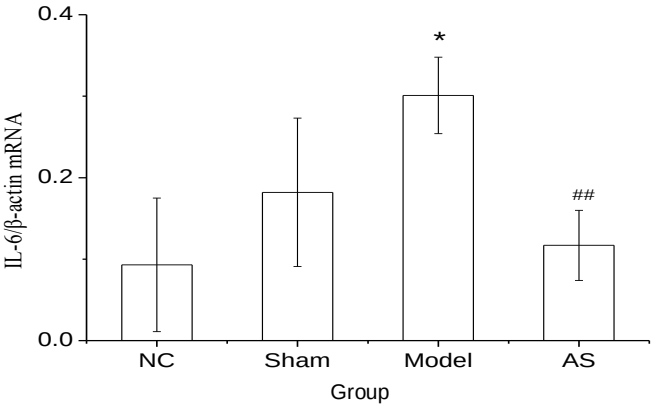

Figure 3. Effect of Angelica sinensis aqueous extract on uterus IL-6/β-actin mRNA expression level. **P*<0.05, compared with sham group; ##*P*<0.01, compared with model group.

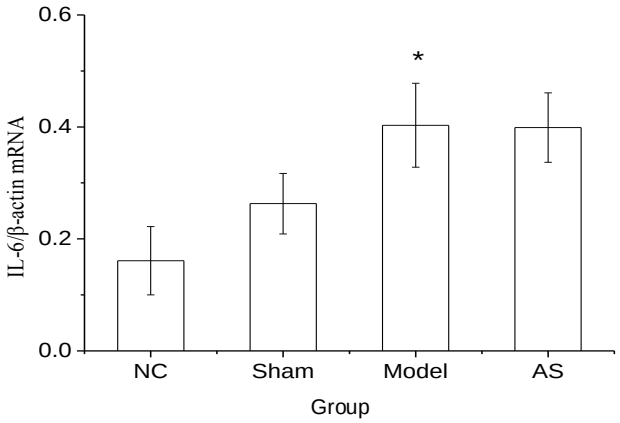

Figure 4. Effect of Angelica sinensis aqueous extract on ovary IL-6/β-actin mRNA expression level. **P*<0.05, compared with sham group.

expression. In the model group, expression level of uterus NF-kB/β-actin mRNA was significantly increased. In the group receiving extracts of *A. sinensis* (0.4 g/kg; po), the gene expression of NF-kB/β-actin mRNA was significantly decreased compared to model group.

The results are shown in Figure 2. There was no significant difference in ovary NF-kB/β-actin mRNA expression. In the model group, expression level of uterus NF-kB/β-actin mRNA was significantly increased. In the group receiving extracts of *A. sinensis* (0.4 g/kg; po), the gene expression of ovary NF-kB/β-actin mRNA was decreased. But the decrease was insignificant.

Interleukin (IL)-6, one of the cytokines, is released from invaded inflammatory cells in various acute phase inflammatory responses (Kishimoto et al., 1992). Its complementary DNA has been cloned by Hirano et al. (1986). Several studies have demonstrated that IL-6 stimulates insulin secretion in HIT-T 15 cells and rat pancreatic islets (Buchard et al., 1990; Sandler et al., 1990; Shimizu et al., 1995). IL-6 may be involved in the regulation of insulin secretion from pancreatic β-cells following inflammatory responses. However, the exact mechanism by which IL-6 stimulates insulin secretion still remains to be established.

Uterus IL-6/β-actin mRNA expression level were presented in Figure 3. Uterus IL-6/β-actin mRNA expression level wasn't significant in the both groups (normal control vs sham), whereas IL-6/β-actin mRNA expression level was observed to significantly increase in model control group compared with untreated sham group. Uterus IL-6/β-actin mRNA expression level was markedly lower in AS group than that in model control group.

Ovary IL-6/β-actin mRNA expression level were presented in Figure 4. Ovary IL-6/β-actin mRNA expression level was not significant in both groups (normal control vs sham), whereas IL-6/β-actin mRNA expression level was observed to significantly increase in model control group compared with untreated sham group. Uterus IL-6/β-actin mRNA expression level was slightly lower in AS group than that in model control group.

IL-6 is an important inflammatory medium and closely associated with systemic inflammatory response. Study shows that increased IL-6 is observed in some gynecological inflammation. TLR4 may stimulate pro-inflammatory cytokines, IL-1o, IL-6 production by PTKs pathway (Zhao et al., 2003). When siRNA was used to block the pathway, IL -6 and IL -10 mRNA expressions was decreased (Matsumura et al., 2010).

Conclusion

Our work suggests that high NF-κB and IL-6 expression induced by bacterial infection in Pelvic inflammation model rats may be reversed by *A. sinensis* aqueous

extract which subsequently decrease inflammation reaction and play its anti inflammatory and immune action.

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