เชียงใหม่สัตวแพทยสาร 2554; 9(2): 93-104

Original article

Effects of dietary supplement of Centella asiatica on serum interleukin-10 and interferon gamma of growing pigs

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Abstract The present study evaluates the effects of *Centella asiatica* L. Urban supplemented in conventional diets on serum levels of interleukin-10 (IL-10) and interferon-gamma (IFN-γ) of growing pigs. Sixteen 8-week old crossbred pigs were divided into four groups of four pigs. Each group was fed *ad libitum* with conventional diets supplemented with either 0% (control), 0.5%, 1%, or 2% pulverized *C. asiatica*. Pigs were bled twice at 12 and 20 weeks of age which corresponded to one and three months of experimental feeding, respectively. The results showed that, after one month of *C. asiatica* feeding, pigs of all experimental groups demonstrated comparable levels of serum IL-10 to control pigs, but pigs fed with 1% and 2% *C. asiatica* demonstrated significantly reduced serum IFN-γ levels. At three months of experimental feeding, pigs fed with 1% and 2% *C. asiatica* showed significantly reduced serum IL-10 and reduced, but not significantly, serum IFN-γ levels. These results indicate that *C. asiatica* has the potential to suppress both anti-inflammatory and pro-inflammatory cytokines. Further study is needed to determine whether these effects of *C. asiatica* have clinical importance in anti-inflammatory treatment. **Chiang Mai Veterinary Journal 2011;9(2): 93-104**

Key Words: *Centella asiatica*, interleukin-10, interferon gamma, serum, swine

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Introduction

The use of medicinal plants in livestock production has become an area of interest for veterinary researchers in recent years. A number of articles have been published with respect to effects of medicinal plants in *in vitro* and *in vivo* settings. Majority of the studies are related to antimicrobial, anti-inflammatory, growth promoting, and immunoenhancing properties of the medicinal plants, their crude extracts, and their purified constituents.[1-5] Main applications of these properties are to improve animal productivity and to reduce or replace the use of antibiotics and chemicals in the production cycle, which relates to food safety.

Centella asiatica L. Urban is a medicinal plant of the Family *Umbelliferae*. It is widely grown in Thailand and is well-known for its anti-oxidative, anti-inflammatory, anti-tumor, wound healing, and cognition improving properties in humans and experimental animals.[6-11] The plant has low or no toxicity even when used at high dose or for long period of time in experimental animals.[9, 12-14] For livestock production, the anti-inflammatory activity of *C. asiatica* is of interest, since it may help reduce clinical symptoms of several infectious diseases, and thereby reduce the use of antimicrobial agents.

Extensive studies regarding the anti-inflammatory activity of *C*. asiatica have been conducted in humans, rats, and mice. These include *in vivo* consumption of and *in vitro* treatment of immune cells with either crude plant extract or active constituents of the plant, i.e. asiatic acid, asiaticoside, madecassic acid, and madecassoside.[10] Oral treatment of rats with crude *C. asiatica extract* led to reduced inflammation in carageenan-paw-induced inflammatory model and acetic acid-induced gastic ulcer model.[11, 15] The rats also showed reduced pruritis and allergy in response to sheep serum and compound 48/80 treatments.[11] Oral treatment of mice with madecassoside decreased collagen-induced arthritis and plasma levels of pro-inflammatory cytokines, i.e. tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) and increased plasma level of anti-inflammatory cytokine, i.e. IL-10.[6] Mice treated orally with crude ethanolic extract of *C. asiatica* also demonstrated higher antibody response to bovine serum albumin.[16]

In vitro treatment of human and murine immune cells with crude *C. asiatica* extract or its active constituents generally leads to decreased expression of inflammatory markers, e.g. IL-1β, IL-6, IL-12, TNF-γ, interferon gamma (IFN-γ), nitric oxide (NO), and prostaglandin E2 (PGE2), and increased expression of anti-inflammatory cytokines, e.g. transforming growth factor-beta (TGF-β) and IL-10.[6-8, 16-20] Mechanisms of these anti-inflammations have been reported to include inhibition of activation of mitogen-activated protein kinases and nuclear factor-kappa B, and of expression of cyclooxygenase-2 and inducible nitric oxide synthase.[15, 19-22]

In pigs, the anti-inflammatory effect of *C. asiatica* has never been studied. In our preliminary *in vitro* study, we reported that treatment of porcine peripheral blood mononuclear cells (PBMC) with crude methanolic extract of *C. asiatica* resulted in up-regulation of both IL-10 and TNF-α expressions in response to concanavalin A (conA) and lipopolysaccharide (LPS) mix stimulation.[23] In the present study, we investigate further the anti-inflammatory potential of *C. asiatica* in pigs *in vivo*. We determine the modulation of serum IL-10 and IFN-γ levels in pigs fed *ad libitum* with diets supplemented with varying percentage of pulverized *C. asiatica*. We aim that this study would suggest potential use of *C. asiatica* for anti-inflammatory purposes in pigs.

Materials and methods

Animals

Sixteen 8-week old crossbred pigs (Landrace-Large White x Duroc) comprising eight castrated males and eight females were used. The pigs were housed at the swine facility, Faculty of Animal Science and Technology, Maejo University (MJU). The animal experiment was approved by the MJU animal care and use committee. All pigs received classical swine fever virus vaccine as a routine vaccination at five weeks of age.

C. asiatica

The aerial parts of *C. asiatica* were collected in Chiang Mai, Thailand during April-May, 2010. The plants were authenticated by the Department of Horticulture, Faculty of Agricultural Production, MJU, and their voucher specimens were deposited at the Faculty of Animal Science and Technology, MJU. The plant materials were dried (50°C) prior to pulverization. The pulverized plants were stored in a dry condition until use.

Experimental design

Pigs were divided into four groups of four pigs (two males and two females per group). Group I to III were fed with conventional diet supplemented with 0.5%, 1%, and 2% of pulverized *C. asiatica*, respectively. Group IV were fed with conventional diet and served as a control group. All groups were fed *ad libitum* throughout the experiment.

IL-10 and IFN-γ

Whole blood samples were collected from jugular vein of all pigs at 12 and 20 weeks of age (corresponding to one and three months after experimental feeding, respectively). Sera were harvested and determined for IL-10 and IFN-γ levels by ELISA (Quantikine®, R&D systems, Minneapolis, MN).

Productive performance

Pigs were weighed at the beginning and the end of the experiment. Their body weight gain as well as total feed intake were collected and used for calculation of average daily gain (ADG), feed conversion ratio (FCR), and average daily feed intake (ADFI).

Statistical analysis

All statistical analyses were performed using the SPSS software version 17 (IBM, Armonk, NY). Mean differences of IL-10, IFN-γ, ADG, FCR, and ADFI levels were tested by one-way analysis of variance, followed by Dunnett's test using mean of group IV as a control group. $P<0.05$ was set as statistically significant level.

Results

IL-10 levels

Pigs fed with *C. asiatica* at either 0.5%, 1%, or 2% demonstrated comparable levels of IL-10 to control pigs after one month of experimental feeding (12-week old) (Fig. 1). After three months of experimental feeding (20-week old), pigs fed with 1% and 2% *C. asiatica* demonstrated significantly reduced IL-10 levels. No difference between reduced IL-10 levels of pigs fed with 1% and 2% *C. asiatica* was observed.

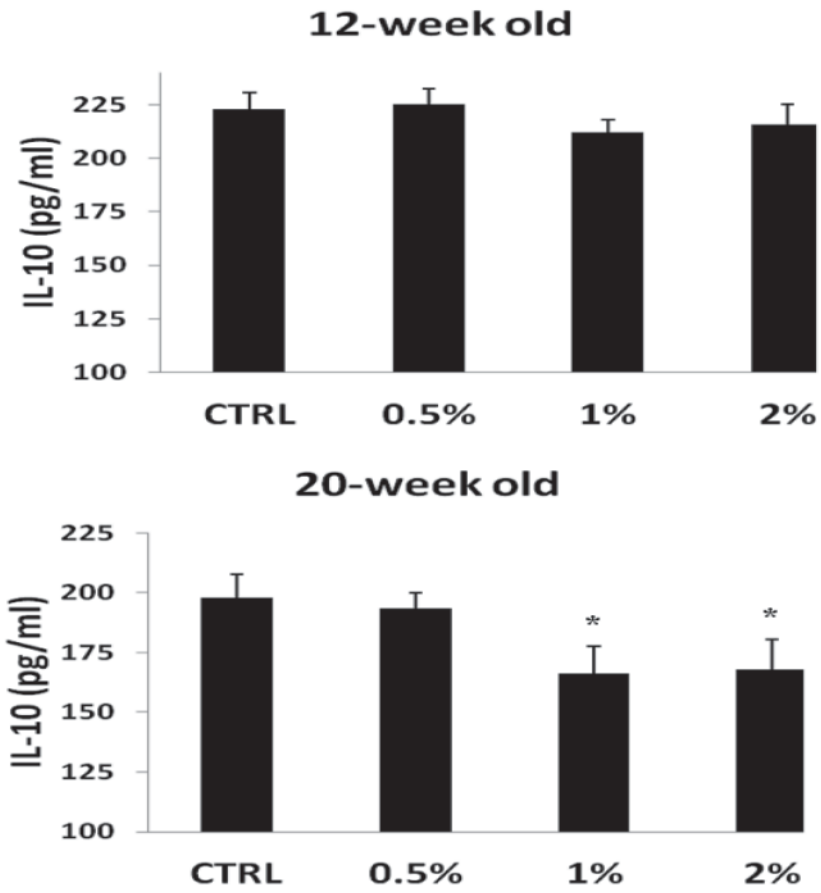

Figure 1. Serum IL-10 levels of pigs fed *ad libitum* with control diet (CTRL), diet supplemented with 0.5%, 1%, or 2% pulverized *C. asiatica*. Pigs were bled twice at 12 and 20 weeks of age (corresponding to one and three months of experimental feeding, respectively). Asterisks indicate significant mean difference from CTRL ($p<0.05$).

IFN-γ levels

After one month of experimental feeding, pigs fed with *C. asiatica* at 1% and 2% demonstrated significantly reduced IFN-γ levels, compared with control pigs (Fig. 2). After three month of experimental feeding, pigs fed with *C. asiatica* at 1% and 2% also demonstrated reduced, but not significantly, IFN-γ levels. No difference between reduced IFN-γ levels of pigs fed with 1% and 2% *C. asiatica* was observed.

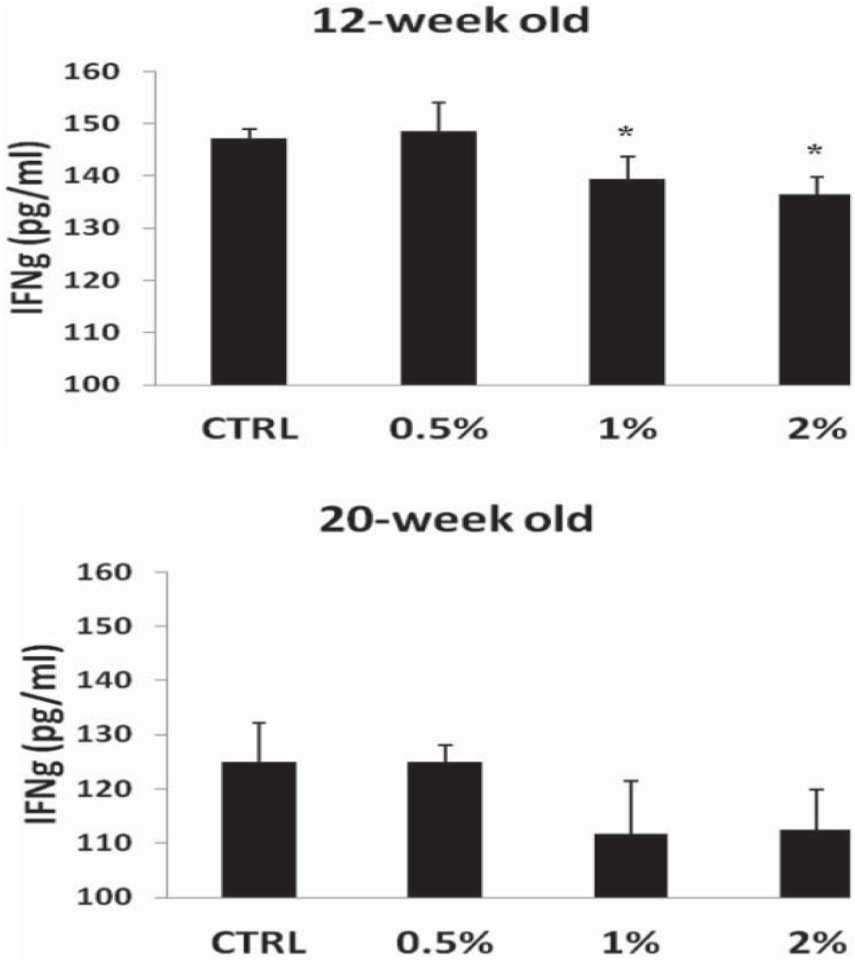

Figure 2. Serum IFNγ levels of pigs fed *ad libitum* with control diet (CTRL), diet supplemented with 0.5%, 1%, or 2% pulverized *C. asiatica*. Pigs were bled twice at 12 and 20 weeks of age (corresponding to one and three months of experimental feeding, respectively). Asterisks indicate significant mean difference from CTRL ($p<0.05$).

Productive performance

Pigs of all *C. asiatica*-treated groups demonstrated comparable ADG, FCR, and ADFI to control pigs (Table.1). No statistical difference of these parameters was observed.

Discussion

The present study evaluated the anti-inflammatory potential of *C. asiatica* in growing pigs. The study determined serum levels of IL-10 and IFN-γ in pigs fed *ad libitum* with diets supplementary

with 0.5%, 1%, or 2% of pulverized *C. asiatica* for three months. The anti-inflammatory potential of *C. asiatica* has never been assessed in swine.

C. asiatica has been reported to possess anti-inflammatory property as assessed in human and murine models. The medicinal plant, either in the form of crude extract or purified active constituents, has been reported to reduce expressions of several inflammatory mediators, e.g. IL-1β, IL-6, IL-12, TNF-α, IFN-γ, NO, and PGE2, and enhance expressions of anti-inflammatory cytokines, e.g. TGF-β and IL-10.[6-8, 16-20] In pigs, the crude methanolic extract of *C. asiatica* has been demonstrated to up-regulate both IL-10 and TNF-α expressions in PBMC in response to conA and LPS mix stimulation.[23]

Pigs fed with *C. asiatica* at any percentage did not demonstrate significant change in serum IL-10 levels after one month of experimental feeding (Fig. 1). The animals, however, demonstrated significantly reduced serum IL-10 levels after they had been fed with 1% and 2% *C. asiatica* for three months. These findings were in contrast to previous findings in humans and other experimental animal species as well as of our *in vitro* study that *C. asiatica* preparations enhanced IL-10 expression. These surprising results were not understood but might be attributed to the effects of various constituents present in pulverized plants or difference in species response to *C. asiatica*. Further investigations are needed and should consider the use of sterile crude plant extracts or active constituents of *C. asiatica* instead of pulverized plants. This may help concentrate anti-inflammatory constituents of the plant. In addition, parenteral route of administration may be used to administer such small amounts of the constituents. These actions together may help elucidate more clearly the effect of *C. asiatica* on porcine IL-10.

Feeding of pigs with 1% and 2% *C. asiatica* resulted in significantly reduced serum IFN-γ levels after one month of experiment (Fig. 2). Continuous feeding of the animals for three months at the same percentage of *C. asiatica* led to reduced, but not statistically significant, serum IFN-γ levels. These findings were compatible with reports in humans, rats, and mice that *C. asiatica* suppressed expressions of several inflammatory mediators including IFN-γ.[6-8, 16-20] These findings suggest that *C. asiatica* might have anti-

Table 1. Effects of *C. asiatica* supplementation on productive performance of swine.

Parameters	% *C. asiatica* supplemented				p-value
	0	0.5	1	2	
Average daily gain (kg)	0.59±0.03	0.54±0.04	0.61±0.02	0.63±0.02	0.26
Feed conversion ratio	2.52±0.11	2.46±0.08	2.36±0.07	2.45±0.08	0.67
Average daily feed intake (kg)	1.55±0.06	1.57±0.01	1.52±0.01	1.60±0.01	0.47

inflammatory potential in the pigs.

In field application, the observations that pigs fed with 1% or 2% pulverized *C. asiatica* demonstrated significantly reduced IFN-γ, but not IL-10 after one month of feeding suggest that supplementation of *C. asiatica* for anti-inflammatory purpose should be done periodically, i.e. supplement at limited period of time, not throughout the entire production period. This strategy of application, however, requires more studies.

The feasibility of *C. asiatica* application in swine production industry is supported by at least two facts that (I) supplementation with *C. asiatica* for three months has no negative impact on pig productive performance (Table 1). Previous studies in rats and mice reported that crude *C. asiatica* extract has high lethal dose 50 (LD50) and causes no acute or chronic toxicity after long-term experimental feeding, which suggest that this medicinal plant is highly safe.[9, 12-14] (II) *C. asiatica* is widely and easily grown in tropical countries, including Thailand, which allows it to have abundant supply and economical cost for swine production industry.

In conclusion, the present study reports the potentials of *C. asiatica* in reducing serum IL-10 and IFN-γ levels in growing pigs which have been fed *ad libitum with* 1% and 2% pulverized plants for three months. The reduction of both IL-10 and IFN-γ makes the anti-inflammatory potential of *C. asiatica* inconclusive in the pigs. Further studies are needed to determine the anti-inflammatory effects of *C. asiatica* preferentially on other inflammatory indicators and in infection models, particularly with bacterial or viral pathogens that cause severe inflammation in the pigs, e.g. *Actinobacillus pleuropneumoniae* and swine influenza virus. In addition, effects of *C. asiatica* on other inflammatory-

related immune parameters, e.g. immunoglobulin subclasses and T lymphocyte subpopulations may be evaluated for more complete information.

Acknowledgement

This work is partially supported by the Thailand Research Fund (grant# DIG5180032) to W. Charerntantanakul, and the Office of Agricultural Research and Extension, Maejo university (grant# MJ.1-53-022) to A. Mekbungwan. The authors thank Silver Wannamook, Sopita Chuaychu, Kanokrat Sirilapa, and Pakatip Wongsakun for technical assistance.

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