대한침구의학회지 제31권 제1호 (2014년 3월) : 131-137
The Acupuncture Vol. 31 No. 1 March 2014 : 131-137
pISSN 1229-1137 eISSN 2287-7797
http://dx.doi.org/10.13045/acupunct.2014013

Original Article

Anti-oxidant and Anti-inflammatory Effects of *Acanthopanacia Cortex* Hot Aqueous Extract on Lipopolysaccharide(LPS) Simulated Macrophages

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[Abstract]

Objectives : This study is to investigate the effects of *Acanthopanacis Cortex* hot aqueous extract on nitric oxide(NO), prostaglandin E2(PGE2) production and DPPH(1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity in macrophages.

Methods : *Acanthopanacis Cortex*(200 g) was heated at 100 ℃ with distilled water(2 L) for 4hrs. The extract was filtered and concentrated to 100 ㎖ using a rotary evaporator and was frozen at -80 ℃, then was freeze-dried. The RAW 264.7 macrophages were subcultured. In order to evaluate cytotoxicity, MTT assay was performed. Experimental groups were divided into five(control, AC 25, 50, 100 and 200 ㎍/㎖) and we measured cytotoxicity. The concentrations of NO were preprocessed by Griess assay. The RAW 264.7 macrophages was pretreated by 10 ㎍/㎖ LPS and experimental groups were divided into five and we measured NO production. The concentrations of PGE_2 were measured by enzyme immunoassay. The RAW 264.7 macrophages was pretreated by 10 ㎍/㎖ LPS. Experimental groups were divided into five and we measured PGE_2 production. Antioxidant activity was measured by the DPPH method. experimental groups were divided into four(AC 25, 50, 100 and 200 ㎍/㎖) and we measured DPPH radical scavenging activity.

Results :

1. Viability of RAW 264.7 macrophages did not significantly decrease in 25, 50 and 100 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract compared to control group.
2. NO production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract compared to control group.
3. PGE_2 production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract compared to control group.
4. DPPH radical scavenging capability of *Acanthopanacis Cortex* hot aqueous extract in RAW 264.7 macrophages had the high level in 100, 200 ㎍/㎖.

Conclusion : According to the results, *Acanthopanacis Cortexx* hot aqueous extract has ability to suppress NO, PGE_2 production and improve DPPH free radical scavenging activity. So *Acanthopanacis Cortex* hot aqueous extract may have an anti-inflammation effect and antioxidant activity.

Key words :
Acanthopanacis Cortex; Anti-inflammation; Antioxidant activity; Hot aqueous extract; Korean medicine

Received : 2014. 02. 18.
Revised : 2014. 02. 27.
Accepted : 2014. 02. 27.
On-line : 2014. 03. 20.

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Ⅰ. Introduction

Acanthopanacis Cortex(AC) is the bark of the roots and stem of acanthopanax tree. In Korean medicine, *Acanthopanacis Cortex* has been widely used for treatment of orthopedic disease[1]. When the tissue have been damaged, inflammatory reaction is shown to combat the pathogen[2].The inflammatory response is a cause of pain in sprains, tendinitis, aponeurositis, gout and osteoarthritis[3].

Previous studies have been reported in the antioxidant[4-6], fat reduction[7,8], antidiabetic[9], anticancer[10,11], increasing cerebral blood flow[12], IL-8 product inhibition[13], blood making, immunodulatory[14] and alcoholysis[15] effect of *Acanthopanacis Cortex*. Likewise, many studies have suggested that the *Acanthopanacis Cortex* might be anti-inflammation effective. But *Acanthopanacis Cortex* was not enough in researching of the anti-inflammation. Therefore, this study is to investigate the effects of *Acanthopanacis Cortex* hot aqueous extract on Nitric Oxide(NO), Prostaglandin E_2(PGE_2) production and DPPH(1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity in macrophages.

Ⅱ. Experimental materials and methods

A. Materials

Acanthopanacis Cortex used in the experiment were purchased from the Omniherb(Kyongbuk, Korea). Extracting method is as follows. *Acanthopanacis Cortex* 200 g mixed with 2 L of distilled water, and boiled for 4 hours at 100 ℃. The extract was filtered with filter paper, and centrifuged at 3,000 xg. And then secondary filtered with 0.03 mm filter paper (Nalgene, New York, USA). The filtrate was concentrated to 100 ml, and frozen at −80 ℃. Freezing solution to be freeze-dried for 7 days through the freezing dryer system(Labconco, USA). 14 g *Acanthopanacis Cortex* extract was obtained. The yield was 7 %.

B. Cell culture

RAW 264.7 macrophages purchased in the ATCC (Manassas, USA) were used in this experiment. RAW 264.7 macrophages were placed in the Dulbecco's modified eagle's medium(DMEM) including 10% Fetal bovine serum(FBS) and were cultivated inside the incubator at 37 ℃ under 5 % CO_2.

C. Measuring cytotoxicity

In order to evaluate cytotoxicity, MTT assay was performed. RAW 264.7 macrophages were cultivated to concentration of 1×10^5/well in a 96 well plate and were cultured for 16 hrs at 37 ℃ under 5 % CO_2. Experimental groups were divided into five(Control, AC 25, 50, 100 and 200 ㎍/㎖). Stable cells were treated in the MTT reagent and absorbance was measured at 570 nm.

D. Measuring NO production

RAW 264.7 macrophages were cultivated to concentration of 1×10^5/well in a 96 well plate and were cultured for 16 hrs at at 37 ℃ under 5 % CO_2. Experimental groups were divided into five(control, AC 25, 50, 100 and 200 ㎍/㎖) and were treated with the 10 ㎍/㎖ Lipopolysaccharide(LPS) reagents. After mixed supernatant 100 ㎕ and Griess reagent 100 ㎕, absorbance was measured at 540 nm. Griess reagent were prepared with 0.1 % naphthylethyleneiamine dihydrochloride 50 ㎕ and 1% sulfanilamide 50 ㎕ dissolved in 5 % H_3PO_4.

E. Measuring PGE_2 production

PGE_2 was measured using the commercial competitive enzyme immunoassay kit(R & D systems,

Minneapolis, USA). Experimental groups were divided into five(control, AC 25, 50, 100 and 200 ㎍/㎖) and were treated with the 10 ㎍/㎖ LPS reagents. Treated RAW 264.7 macrophages were cultured for 18 hrs at at 37 ℃ under 5 % CO_2. Each culture fluid 100 ㎖ loaded at goat anti-mouse coated 96 well plate, and then mixed primary antibody solution 50 ㎕ and PGE_2 conjugate 50 ㎕. It was stabilized for overnight at 4 ℃. Treated culture fluid mixed substrate solution 200 ㎕ and waiting for 5~20 minutes, and then mix the stop solution 50 ㎕. Absorbance was measured at 450 nm.

F. Measuring the antioxidative effect

In order to investigate the antioxidative ability of *Acanthopanacis Cortex*, DPPH free radical scavenging capability was measured. RAW 264.7 macrophages were cultivated to concentration of 1×10^5/well in a 96 well plate and were cultured for 16 hrs at at 37 ℃ under 5 % CO_2. Experimental groups were divided into four(AC 25, 50, 100 and 200 ㎍/㎖) and were diluted with MeOH(Methanol) and diluent 50 ㎕ mixed with 0.15 mM DPPH(Sigma, USA) 80 ㎕ in 96 well plate. Isolate the light at room temperature for 3 minutes. Absorbance was measured using the microplate reader(Tecan, Italy) at 520 nm. DPPH free radical scavenging capability was evaluated in the following way.

$$\text{DPPH free radical scavenging activity}(\%) = \frac{Absorbance\,of\,Control - \text{Experimental}}{Absorbance\,of\,Control} \times 100$$

G. Statistical analysis

With SPSS Window program(Ver. 10.0), experimental result were processed in terms of averages ± standard deviations. Statistical significance was confirmed by using Student's *t*-test, the level of significance was $p<0.05$.

III. Result

a. Viability of RAW 264.7 macrophages did not significantly decrease in 25, 50 and 100 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract compared to control group.

b. NO production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract compared to control group.

c. PGE_2 production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract compared to control group.

d. DPPH radical scavenging capability of *Acantho-panacis Cortex* hot aqueous extract in RAW 264.7 macrophages had the high level in 100, 200㎍/㎖.

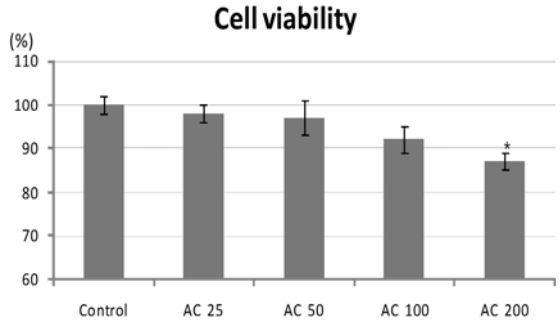

Fig. 1. This graph describes that concentrations of *Acanthopanacis Cortex* hot aqueous extract make a difference in the viability of RAW 264.7 macrophage

Control : non-treated group.
AC 25 : 25 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 50 : 50 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 100 : 100 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 200 : 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
Values are represented as mean ± SD.
* : statistically significant difference from the normal group, as determined by the student's *t*-test as $p < 0.05$.

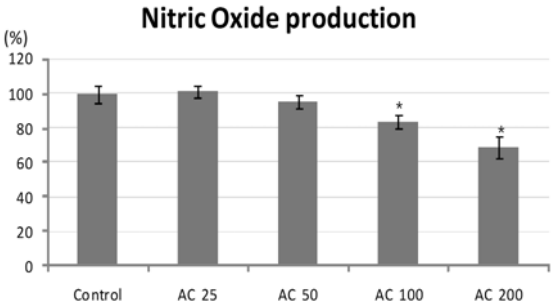

Fig. 2. This graph describes that concentrations of *Acanthopanacis Cortex* hot aqueous extract make a difference in the NO production of RAW 264.7 macrophages

Control : 10 ㎍/㎖ LPS treated group
AC 25 : 10 ㎍/㎖ LPS and 25 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group
AC 50 : 10 ㎍/㎖ LPS and 50 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 100 : 10 ㎍/㎖ LPS and 100 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 200 : 10 ㎍/㎖ LPS and 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
Values are represented as mean ± SD.
* : statistically significant difference from the control group, as determined by the student's *t*-test as $p < 0.05$.

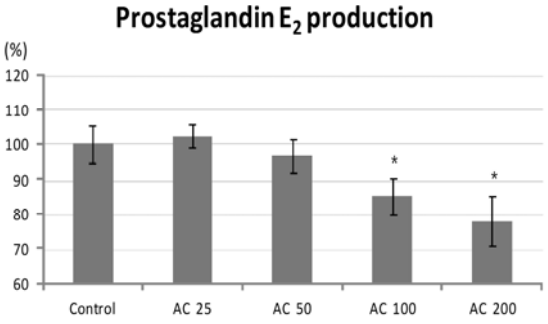

Fig. 3. This graph describes that concentrations of *Acanthopanacis Cortex* hot aqueous extract make a difference in the PGE2 production of RAW 264.7 macrophages

Control : 10 ㎍/㎖ LPS treated group.
AC 25 : 10 ㎍/㎖ LPS and 25 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 50 : 10 ㎍/㎖ LPS and 50 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 100 : 10 ㎍/㎖ LPS and 100 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
AC 200 : 10 ㎍/㎖ LPS and 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group.
Values are represented as mean ± SD.
* : statistically significant difference from the control group, as determined by the student's *t*-test as $p < 0.05$.

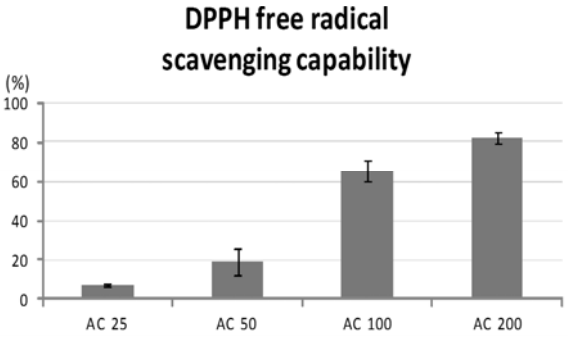

Fig. 4. This graph describes that concentrations of *Acanthopanacis Cortex* hot aqueous extract make a difference in the DPPH free radical scavenging capability of RAW 264.7 macrophages

AC 25 : 25 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group
AC 50 : 50 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group
AC 100 : 100 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group
AC 200 : 200 ㎍/㎖ *Acanthopanacis Cortex* hot aqueous extract treated group
Values are represented as mean ± SD.

IV. Discussion

The effect of *Acanthopanacis Cortex* stated in Sinnongbonchokyung as follows. *Acanthopanacis Cortex* can be used in diseases of the joints, skin, internal[16]. In Seong's study[17], exhibit that *Acanthopanacis Cortex* is effective in rheumatic pains, myalgia and neuralgia. In Lee's study[18], show that *Acanthopanacis Cortex* is effective in anti- inflammation and antitumor.

In terms of oriental medicine, the inflammatory response is competing vital force and bad factor. Vital force means resisting power against disease. Bad factor means cause of disease[19]. In terms of pathology, inflammatory response is process of restoring the damaged area. It is a variety of factors are involved. The reaction is active and, in this process, tissue damage is minimized[20]. Clinically, inflammatory response appear of redness, swelling, fever, pain and functional disability symptoms[3].

NO is freeradical. It is the transfer substances of cardiovascular, nervous system and the immune system. Its role is Intracellular homeostasis, transport of neurotransmitters, anticancer[21].

But Its excessive production is to cause another inflammatory and tissue damage. Persistent inflammatory response can result in myocarditis, chronic arthritis, glomerulonephritis, insulin dependent diabetes mellitus, and irritable bowel[22]. Therefore in recent various study, looking for ways to treat inflammation by effectively suppress the production of NO[23-25].

PGE_2 is also an important inflammatory mediator. It is synthesized by COX-2, and activated by macrophages. COX-2 not expressed in the resting state, but expression is induced when the inflammatory stimulation. Large excess of PGE_2 is lasting inflammatory reaction to cause rubefaction, edema, stiffness and pain[26,27].

In the human body, oxidation promoting substance and oxidation inhibitors are in balance. When this balance was broken, the oxidative stress was generated and protein is decomposed, DNA synthesis is inhibited. Consequently, the harmful action of the cells and organs[28]. Reactive oxygen species(ROS) and reactive nitrogen species(RNS) are involved in this oxidative damage.

Free radical, such as superoxide($O2^-$), nitric oxide(NO) and hydroxyl radical(HO^-), and oxygen-derived species, such as singlet oxygen($^1O2$) and hydrogen peroxide(H2O2) are belong here[29].

DPPH is free radical. It is purple, and has a light-absorbing at 520 nm. Generally it switch to DPPH-H and discolored by the proton-radical scavenger in various antioxidant mechanisms. Therefore, the results can be verified easily by the naked eye. In addition, it has the advantage of very stable at organic solvents such as alcohol[30].

Result in cytotoxicity about *Acanthopanacis Cortex* hot aqueous extract is that the control group 100 ± 2 %, *Acanthopanacis Cortex* hot aqueous extract 25 ㎍/㎖ group 98 ± 2 %, 50 ㎍/㎖ group 97 ± 4 %, 100 ㎍/㎖ group 92 ± 3 % and 200 ㎍/㎖ group 87 ± 2 %. This result indicates that there is no significant toxicity up to 100 ㎍/㎖.

Result of investigation NO production rate of the RAW 264.7 macrophages were treated *Acanthopanacis Cortex* hot aqueous extract is that the control group 100.00 ± 5.00 %, *Acanthopanacis Cortex* hot aqueous extract 25 ㎍/㎖ group 101.52 ± 3.42 %, 50 ㎍/㎖ group 95.42 ± 4.06 %, 100 ㎍/㎖ group 83.62 ± 3.84 % and 200 ㎍/㎖ group 68.97 ± 6.36 %. The NO production rate was increased within an error range at *Acanthopanacis Cortex* hot aqueous extract 25 ㎍/㎖ group. And NO Production rate was significantly decreased at *Acanthopanacis Cortex* hot aqueous extract 100 ㎍/㎖ and 200 ㎍/㎖ group. In Yoon's study[15], shows that Acanthopanacis senticosus Cortex hot aqueous extract inhibits the NO generation dose-dependently.

Result of investigation PGE2 production rate of the RAW 264.7 macrophages were treated *Acanthopanacis Cortex* hot aqueous extract is that the control group 100.00 ± 5.14 %, *Acanthopanacis Cortex* hot aqueous extract 25 ㎍/㎖ group 102.35 ± 3.35 %, 50 ㎍/㎖ group 96.74 ± 4.72 %, 100 ㎍/㎖ group 85.09 ± 5.16 % and 200 ㎍/㎖ group 77.91 ± 7.24 %. The PGE2 production rate was increased within an error range at *Acanthopanacis Cortex* hot aqueous extract 25 ㎍/㎖ group. And PGE2 production rate was significantly decreased at *Acanthopanacis Cortex* hot aqueous extract 100 ㎍/㎖ and 200 ㎍/㎖ group.

Result of investigation scavenging ability of the RAW 264.7 macrophages were treated *Acanthopanacis Cortex* hot aqueous extract is that the *Acanthopanacis Cortex* hot aqueous extract 25 ㎍/㎖ group 6.99 ± 0.98 %, 50 ㎍/㎖ group 19.13 ± 6.84 %, 100 ㎍/㎖ group 65.50 ± 5.41 % and 200 ㎍/㎖ group 82.60 ± 2.92 %. *Acanthopanacis Cortex* hot aqueous extract was 50 % or more of high erasure rate at 100 ㎍/㎖ and 200 ㎍/㎖ group. In Im's study[4] *Acanthopanacis Cortex* Ethanol extract has antioxidant effect in 20 % and 40 % concentration.

Analyzing the results of this experiments, *Acanthopanacis Cortex* hot aqueous extract has decreased NO and PGE2 production rate and scavenging DPPH radical in 50 ㎍/㎖, 100 ㎍/㎖ and 200 ㎍/㎖ concentration. Therefore, *Acanthopanacis Cortex* hot aqueous extract is believed to have an anti-inflammatory and antioxidant effect. But in 25 ㎍/㎖ concentration, increased NO and PGE2 production rate slightly. So it is possible that Acanthopanacis senticosus Cortex hot aqueous increase inflammatory

reactions in low concentration. Therefore, various studies on Acanthopanacis senticosus Cortex about concentration, extraction method and clinical study should be continued.

V. Conclusion

According to the results, *Acanthopanacis Cortex* hot aqueous extract has ability to suppress NO, PGE2 production and improve DPPH free radical scavenging activity. So *Acanthopanacis Cortex* hot aqueous extract may have an anti-inflammation effect and antioxidant activity.

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