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

Na Young Jo and Jeong Du Roh*

Department of Acupuncture & Moxibustion Medicine, Je-Cheon Hospital of Traditional Korean Medicine, Semyung University

[Abstract]

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

Methods: *Acanthopanacia Cortex* (200 g) was heated at 100 ℃ with distilled water (2 L) for 4 hrs. The extract was filtered and concentrated to 100 ml 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 µg/ml) and we measured cytotoxicity. The concentrations of NO were preprocessed by Griess assay. The RAW 264.7 macrophages was pretreated by 10 µg/ml LPS and experimental groups were divided into five and we measured NO production. The concentrations of PGE2 were measured by enzyme immunoassay. The RAW 264.7 macrophages was pretreated by 10 µg/ml LPS. Experimental groups were divided into five and we measured PGE2 production. Antioxidant activity was measured by the DPPH method, experimental groups were divided into four (AC 25, 50, 100 and 200 µg/ml) and we measured DPPH radical scavenging activity.

Results:
1. Viability of RAW 264.7 macrophages did not significantly decrease in 25, 50 and 100 µg/ml *Acanthopanacia Cortex* hot aqueous extract compared to control group.
2. NO production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 µg/ml *Acanthopanacia Cortex* hot aqueous extract compared to control group.
3. PGE2 production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 µg/ml *Acanthopanacia Cortex* hot aqueous extract compared to control group.
4. DPPH radical scavenging capability of *Acanthopanacia Cortex* hot aqueous extract in RAW 264.7 macrophages had the high level in 100, 200 µg/ml.

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

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

*Corresponding author: Department of Acupuncture & Moxibustion Medicine, Je-Cheon Hospital of Traditional Korean Medicine, Semyung University, 65, Semyung-ro, Jecheon-si, Chungcheongbuk-do, 390-711, Republic of Korea
Tel : +82-43-649-1816  E-mail : wsrohmio@hanmail.net

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

The Acupuncture is the Journal of Korean Acupuncture & Moxibustion Medicine Society. (http://www.TheAcupuncture.or.kr) Copyright © 2014 KAMMS, Korean Acupuncture & Moxibustion Medicine Society. All rights reserved.
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, aponeurosis, 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 Omniherbs (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 °C. 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 °C. 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 °C 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 °C under 5 % CO\(_2\). Experimental groups were divided into five (Control, AC 25, 50, 100 and 200 µg/ml), 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 37 °C under 5 % CO\(_2\). Experimental groups were divided into five (control, AC 25, 50, 100 and 200 µg/ml) and were treated with the 10 µg/ml Lipopolysaccharide (LPS) reagents. After mixed supernatant 100 µl and Griess reagent 100 µl, absorbance was measured at 540 nm. Griess reagent were prepared with 0.1 % naphthyl ethyleneamine dihydrochloride 50 µl and 1% sulfanilamide 50 µl dissolved in 5 % H\(_3\)PO\(_4\).

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 μg/ml) and were treated with the 10 μg/ml LPS reagents. Treated RAW 264.7 macrophages were cultured for 18 hrs at 37 ℃ under 5 % CO2. Each culture fluid 100 ml loaded at goat anti-mouse coated 96 well plate, and then mixed primary antibody solution 50 μl and PGE2 conjugate 50 μl. It was stabilized for overnight at 4 ℃. Treated culture fluid mixed substrate solution 200 μl and waiting for 5~20 minutes, and then mix the stop solution 50 μl. 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 37 ℃ under 5 % CO2. Experimental groups were divided into four (AC 25, 50, 100 and 200 μg/ml) and were diluted with MeOH(Methanol) and diluent 50 μl mixed with 0,15 mM DPPH(Sigma, USA) 80 μl 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.

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

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 μg/ml *Acanthopanacis Cortex* hot aqueous extract compared to control group,

b. NO production in LPS–stimulated RAW 264,7 macrophages significantly inhibited in 100, 200 μg/ml *Acanthopanacis Cortex* hot aqueous extract compared to control group,

c. PGE2 production in LPS–stimulated RAW 264,7 macrophages significantly inhibited in 100, 200 μg/ml *Acanthopanacis Cortex* hot aqueous extract compared to control group,

d. DPPH radical scavenging capability of *Acanthopanacis Cortex* hot aqueous extract in RAW 264,7 macrophages had the high level in 100, 200μg/ml.
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 µg/ml LPS treated group
AC 25 : 10 µg/ml LPS and 25 µg/ml Acanthopanacis Cortex hot aqueous extract treated group
AC 50 : 10 µg/ml LPS and 50 µg/ml Acanthopanacis Cortex hot aqueous extract treated group,
AC 100 : 10 µg/ml LPS and 100 µg/ml Acanthopanacis Cortex hot aqueous extract treated group.
AC 200 : 10 µg/ml LPS and 200 µg/ml 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 µg/ml LPS treated group,
AC 25 : 10 µg/ml LPS and 25 µg/ml Acanthopanacis Cortex hot aqueous extract treated group,
AC 50 : 10 µg/ml LPS and 50 µg/ml Acanthopanacis Cortex hot aqueous extract treated group,
AC 100 : 10 µg/ml LPS and 100 µg/ml Acanthopanacis Cortex hot aqueous extract treated group,
AC 200 : 10 µg/ml LPS and 200 µg/ml 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 \( p < 0.05 \).

IV. Discussion

The effect of Acanthopanacis Cortex stated in Sinnongbonchokyung as follows. Acanthopanacis Cortex can be used in diseases of the joints, skin, internal16). In Seong’s study17), exhibit that Acanthopanacis Cortex is effective in rheumatic pains, myalgia and neuralgia. In Lee’s study18), show that Acanthopanacis Cortex is effective in anti-inflammatory 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 disease19). 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 minimized20). Clinically, inflammatory response appear of redness, swelling, fever, pain and functional disability symptoms3).

NO is freeradical, It is the transfer substances of cardiovascular, nervous system and the immune system. Its role is Intracellular homeostasis, transport of neurotransmitters, anticancer21).
Anti-oxidant and Anti-inflammatory Effects of Acanthopanacis Cortex Hot Aqueous Extract on Lipopolysaccharide(LPS) Simulated Macrophages

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 syndrome. Therefore, in recent years, various studies have looked for ways to treat inflammation by effectively suppress the production of NO (23-25).

PGE2 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 PGE2 is lasting inflammation 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 (O2) 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 μg/ml group 98 ± 2 %, 50 μg/ml group 97 ± 4 %, 100 μg/ml group 92 ± 3 % and 200 μg/ml group 87 ± 2 %. This result indicates that there is no significant toxicity up to 100 μg/ml.

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 μg/ml group 101,52 ± 3,42 %, 50 μg/ml group 95,42 ± 4,06 %, 100 μg/ml group 83,62 ± 3,84 % and 200 μg/ml group 68,97 ± 6,36 %. The NO production rate was increased within an error range at Acanthopanacis Cortex hot aqueous extract 25 μg/ml group. And NO Production rate was significantly decreased at Acanthopanacis Cortex hot aqueous extract 100 μg/ml and 200 μg/ml group. In Yoon's study (31), 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 μg/ml group 102,35 ± 3,35 %, 50 μg/ml group 96,74 ± 4,72 %, 100 μg/ml group 85,99 ± 5,16 % and 200 μg/ml group 77,91 ± 7,24 %. The PGE2 production rate was increased within an error range at Acanthopanacis Cortex hot aqueous extract 25 μg/ml group. And PGE2 production rate was significantly decreased at Acanthopanacis Cortex hot aqueous extract 100 μg/ml and 200 μg/ml 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 μg/ml group 6,99 ± 0,98 %, 50 μg/ml group 19,13 ± 6,84 %, 100 μg/ml group 65,50 ± 5,41 % and 200 μg/ml group 82,60 ± 2,92 %. Acanthopanacis Cortex hot aqueous extract was 50 % or more of high erasure rate at 100 μg/ml and 200 μg/ml group. In Im's study (32) 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 μg/ml, 100 μg/ml and 200 μg/ml concentration. Therefore, Acanthopanacis Cortex hot aqueous extract is believed to have an anti-inflammatory and antioxidant effect. But in 25 μg/ml 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 Acanthopanax senticosus Cortex about concentration, extraction method and clinical study should be continued.

V. Conclusion

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

VI. References

2. Chapter of The Korea Society of Pathologists, Summarize Pathology, Jungmoongak. 2008 : 81
3. Song KY, Kim MK, Ji GK, Core Pathology, Korea medical books, 2013 : 45, 61–3, 97