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Original Articles

유산균발효애엽이 독성물질들로 유발된 대식세포의 일산화질소생성 감소에 미치는 영향

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Effect of Lactobacillus pentosus-Fermented Artemisiae Argi Folium on Nitric Oxide Production of Macrophage impaired with Various Toxicants

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ABSTRACT

Objectives : The purpose of this study is to investigate the effect of Water Extract from Lactobacillus pentosus-fermented ARTEMISIAE ARGI FOLIUM (AFL) on nitric oxide production of mouse macrophage Raw 264.7 cells impaired by various toxicants such as gallic acid, EtOH, nicotine, acetaminophen, and acetaldehyde.

Methods : ARTEMISIAE ARGI FOLIUM was fermented with Lactobacillus pentosus and extracted by water. Nitric oxide production of mouse macrophage Raw 264.7 cells was measured by Griess reagent assay. Examined concentrations of AFL were 10, 50, 100, 200, 400 ug/mL.

Results : The results of the experiment are as below.

1. AFL at the concentration of 400 ug/mL significantly recovered nitric oxide production which was reduced by gallic acid (100 uM) in Raw 264.7 cells.
2. AFL at the concentration of 200, 400 ug/mL significantly recovered nitric oxide production which was reduced by EtOH (100 uM) in Raw 264.7 cells.
3. AFL at the concentration of 400 ug/mL significantly recovered nitric oxide production which was reduced by nicotine (1mM) in Raw 264.7 cells.
4. AFL at the concentration of 200, 400 ug/mL significantly recovered nitric oxide production which was reduced by acetaminophen(2 mM) in Raw 264.7 cells.
5. AFL at the concentration of 200, 400 ug/mL significantly recovered nitric oxide production which was reduced by acetaldehyde (200 uM) in Raw 264.7 cells.

Conclusions : AFL could be supposed to have the immune-enhancing activity related with nitric oxide production of macrophage impaired by various toxicants.

목적:이 연구의 목적은 유산균발효애엽 물추출물이 에탄올 등으로 약화된 마우스 대식세포의 NO 생성에 미치는 영향을 조사하는 것이다.

방법:애엽을 유산균으로 발효시켜 시료(AFL)를 만들고 만들어진 시료를 10, 50, 100, 200, 400 ug/mL의 농도로 에탄올, 갈릭산, 아세트아미노펜, 아세트알데히드, 니코틴과 함께 24시간동안 마우스 대식세포에 처리한 후 세포배양액을 채취, NO 생성정도를 측정하여 비교하였다.

결과:1. AFL은 400 ug/mL의 농도에서 갈릭산에 의한 마우스 대식세포의 NO생성억제를 유의하게 회복시켰다.
2. AFL은 200, 400 ug/mL의 농도에서 에탄올에 의한 마우스 대식세포의 NO생성억제를 유의하게 회복시켰다.
3. AFL은 400 ug/mL의 농도에서 니코틴에 의한 마우스 대식세포의 NO생성억제를 유의하게 회복시켰다.
4. AFL은 200, 400 ug/mL의 농도에서 아세트아미노펜에 의한 마우스 대식세포의 NO생성억제를 유의하게 회복시켰다.
5. AFL은 200, 400 ug/mL의 농도에서 아세트알데히드에 의한 마우스 대식세포의 NO생성억제를 유의하게 회복시켰다.

결론:유산균발효애엽추출물(AFL)은 에탄올, 갈릭산, 니코틴, 아세트알데히드, 아세트아미노펜 등에 의해 약화된 대식세포의 NO생성을 회복시킴으로서 다양한 독성물질에 의하여 약화되는 대식세포의 항병능력을 회복시키는 면역강화물질로 개발될 수 있을 것이다.

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I. Introduction

Artemisiae Argi Folium (AAF) is the dried leaf of Artemisia argyi Levl. et Vanf. (family Compositae) and used to treat various diseases such as menorrhagia, vaginal bleeding during pregnancy, irregular menstruation, pain with a cold feeling in the lower abdomen, sterility related with uterine malfunction, and leukorrhagia in oriental medicine[1,2]. Besides, the medicinal decoction of Artemisiae Argi Folium is used to care skin diseases like skin eczema[3] and itching in East Asia including Korea, China, and Japan.

Fermentation by *Lactobacillus* sp. is known to be beneficial for food production and preservation[4]. Kimchi, a famous korean traditional food, is also made through fermentation by *Lactobacillus* sp[5].

Nitric oxide (NO) is a reactive radical molecule produced from guanidino nitrogen of L-arginine, which is oxidized by NO synthase (NOS)[6]. NO is produced by a variety of cell types including macrophage and monocyte and essential for host innate immune response to pathogens such as bacteria, viruses, fungi, and parasites[7].

Macrophage is a major immune cell to remove pathogens such as bacteria and virus through secreting NO, cytokines, chemokines, growth factors, and various enzymes[8].

Although many studies have examined pharmacological activities of AAF[9-12], immunological effects of fermented AAF is not yet studied sufficiently.

In this study, we fermented Artemisiae Argi Folium by *Lactobacillus pentosus* and investigated effects of *Lactobacillus pentosus*-fermented ARTEMISIAE ARGI FOLIUM (AFL) on NO production of mouse macrophage impaired by Gallic acid (GA), EtOH, Nicotine, Acetaminophen(AAP), and Acetaldehyde(AC).

II. Materials and method

1. Preparation of AFL

AAF was purchased from Omniherb (Daegu, Korea) in October 2008. A voucher specimen (No. 2008-10-0014) was deposited at the College of Oriental Medicine, Kyungwon University Herbarium. AAF (50g) was extracted with 1000 mL of boiling water for 150 min, filtered, and then lyophilized (yield: AAF, 12%). These water extracts (3.0g; pH 5.44) were suspended in 2.2 mL of water including α-herbzyme (3g, Hankuk Hyoso, Korea) and incubated for 96 h at 37℃ with *Lactobacillus pentosus.* Following an additional incubation for 20 min at 60℃, extracts were concentrated under vacuum. Extract was named as AFL by abbreviating both 'Artemisiae Argi Folium' and *'Lactobacillus pentosus'.* The powdered extract (AFL; pH 5.42) was dissolved in normal saline and then filtered through a 0.22 um syringe filter before use.

2. Cell line

RAW 264.7 mouse macrophage cell line was purchased from the Korea Cell Line Bank (Seoul, Korea).

3. Cell culture

Cells were cultured in DMEM (Sigma, USA) supplemented with 10% FBS (Sigma, USA) containing 100U/mL of penicillin and 100 ug/mL of streptomycin. Cells incubated at 37℃ in a 5% CO_2 humidified incubator[13-16]. Briefly, cells (1×10^4 cells/well) were seeded in a 96 well plate and treated with toxicants (GA, EtOH, Nicotine, AAP, and AC) and AFL. After 24h incubation, the supernatant from each well was taken for the immunological assay.

4. NO production

NO concentration in the cultured medium was determined via the Griess reaction[17]. Specifically, 60 uL of supernatant from each well was taken after 24h incubation and mixed with 60uL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water) in a separate 96well plate. After 15min at room temperature, the optical density was determined at 540nm with a microplate reader(Bio-Rad, Hercules, CA, USA). NO production was calculated and compared as below.

Productions of nitric oxide(%) = 100×AT/AC

AC- absorbance of control

AT- absorbance of tested extract solution.

5. Statistical analysis

The results shown are summarized from at least five independent experiments and presented as the mean±S.D. Significant differences were examined using an analysis of variance (ANOVA) and a Student's t-test with SPSS (version 11.0).

III. Results

1. Effect of AFL on NO production of Raw 264.7 cells impaired by GA

AFL at the concentration of 400 ug/mL increased significantly (P<0.05) NO production of Raw 264.7 cells reduced by GA (100uM) (Fig. 1).

2. Effect of AFL on NO production of Raw 264.7 cells impaired by EtOH

AFL at the concentration of 200 and 400ug/mL increased significantly (P<0.05) NO production of Raw 264.7 cells reduced by EtOH (100uM) (Fig.2).

3. Effect of AFL on NO production of Raw 264.7 cells impaired by Nicotine

AFL at the concentration of 400ug/mL increased significantly (P<0.05) NO production of Raw 264.7 cells reduced by Nicotine (1mM) (Fig. 3)

4. Effect of AFL on NO production of Raw 264.7 cells impaired by AAP

AFL at the concentration of 200 and 400 ug/mL increased significantly (P<0.05) NO production of Raw 264.7 cells reduced by AAP (2mM) (Fig.4).

5. Effect of AFL on NO production of Raw 264.7 cells impaired by AC

AFL at the concentration of 200 and 400ug/mL increased significantly (P<0.05) NO production of Raw 264.7 cells reduced by AC (200uM) (Fig.5).

IV. Discussion

In recent researches, pharmacochemical activities of *Artemisia* species including *Artemisia argyi* were much reported[18-23]. Of those, ARTEMISIAE ARGI FOLIUM water extract was already known to increase NO production in Raw 264.7 cells impaired by EtOH, Nicotine, AAP, and AC[24]. And *Sacchromyces cerevisiae*-fermented ARTEMISIAE ARGI FOLIUM water extract was also reported to upregulate NO production in Raw 264.7 cells impaired by various toxicants[25].

Recently, fermentation with various herbal drugs have been tried for enhancing pharmacological effect and safety of herbal drug. But studies for

effect of *Lactobacillus pentosus*-fermented ARTEMISIAE ARGI FOLIUM (AFL) on macrophage's immuno activity is not elucidated thoroughly.

Instead of pathologic organisms, Various toxicants such as GA, ethanol, Nicotine, AAP, and AC could make macrophage's immune function weaken through decreasing NO production from macrophage. Decreased NO production of macrophage might be the first step for a living system to be attacked by dangerous pathogens. Thus, preserving NO production of macrophage is important for protecting host from invading pathogens. In this experiments, though at high concentrations (200 and 400ug/mL), AFL showed restoring effects on mouse macrophage cell's NO production decreased by GA, ethanol, Nicotine, AAP, and AC. Specially, AFL increased viabilities of Raw 264.7 cells impaired by AAP.

These results mean that AFL could be developed as one of the immune-enhancing herbal medicines related with preserving NO production of macrophage.

V. Conclusions

In this study, we investigate the effects of AFL on NO production of Raw 264.7 mouse macrophage cells impaired by various toxicants such as GA, EtOH, Nicotine, AAP, and AC. Cells were incubated with each toxicant and AFL (10, 50, 100, 200, 400ug/mL) for 24h. AFL show effects as below.

1. AFL increased NO production of Raw 264.7 cells impaired by GA at the concentration of 400 ug/mL significantly ($p<0.05$).

2. AFL increased NO production of Raw 264.7 cells impaired by EtOH at the concentration of 200 and 400 ug/mL significantly ($p<0.05$).

3. AFL increased NO production of Raw 264.7 cells impaired by Nicotine at the concentration of 400 ug/mL significantly ($p<0.05$).

4. AFL increased NO production of Raw 264.7 cells impaired by AAP at the concentration of 200 and 400 ug/mL significantly ($p<0.05$).

5. AFL increased NO production of Raw 264.7 cells impaired by AC at the concentration of 200 and 400 ug/mL significantly ($p<0.05$).

Based on these results, it could be supposed that AFL at high concentrations could preserve macrophage's immune-activity related with NO expression damaged by various toxicants such as GA, EtOH, Nicotine, AAP, and AC. The continued research for exact mechanism for immuno-enhancing activity of AFL related with macrophage remains to be accomplished.

VI. Acknowledgements

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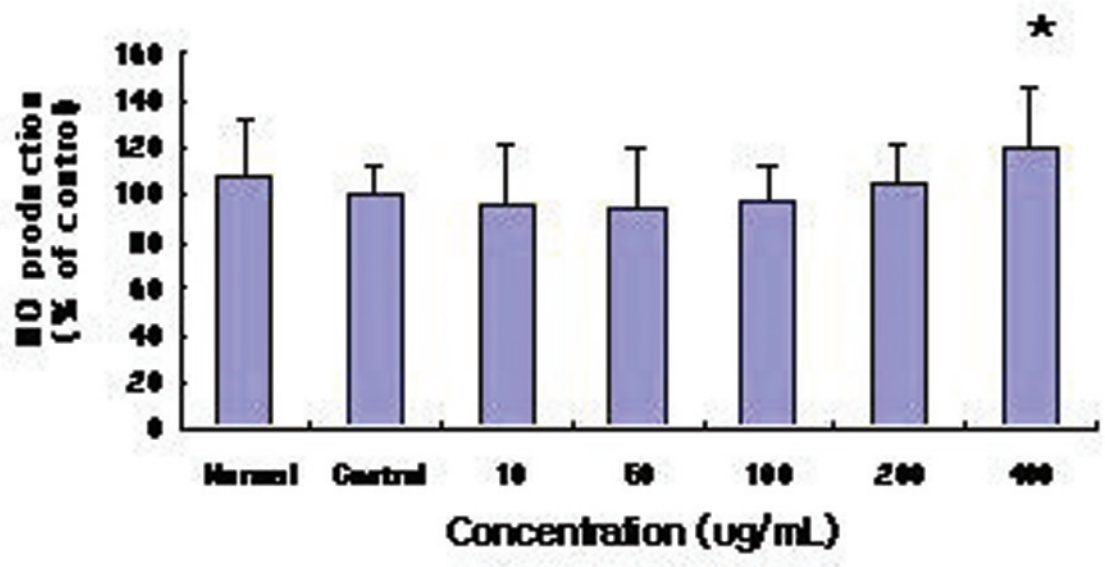

Fig. 1.: Effect of AFL on nitric oxide (NO) production of Raw 264.7 mouse macrophage cells impaired by Gallic acid (GA). NO production was determined using Griess reagent assay. Cells were incubated with GA (100 uM) and AFL (10, 50, 100, 200, 400 ug/mL) for 24 h. Results are represented as mean ± S.D. Normal : Not treated with GA. Control : Treated with GA only.
* represents $P < 0.05$ compared to the control.

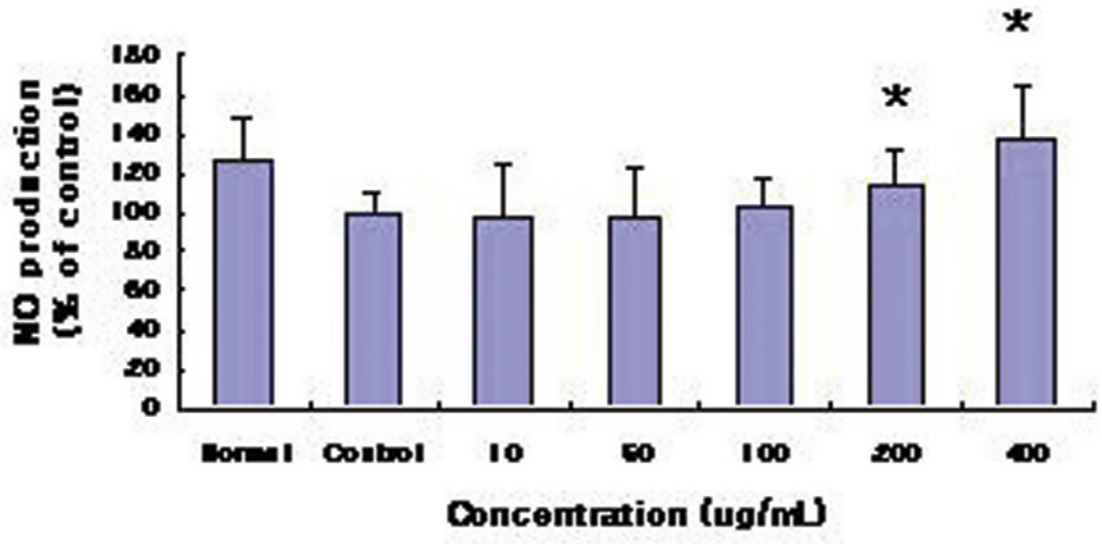

Fig. 2.: Effect of AFL on nitric oxide (NO) production of Raw 264.7 mouse macrophage cells impaired by EtOH. NO production was determined using Griess reagent assay. Cells were incubated with EtOH (100 uM) and AFL (10, 50, 100, 200, 400 ug/mL) for 24 h. Results are represented as mean ± S.D. Normal : Not treated with EtOH. Control : Treated with EtOH only.
* represents $P < 0.05$ compared to the control.

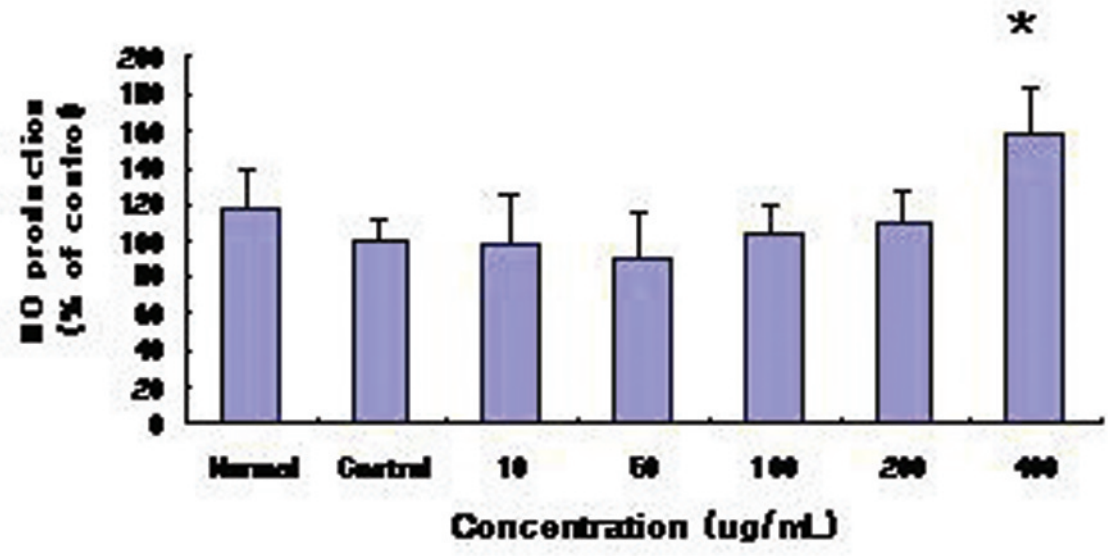

Fig. 3.: Effect of AFL on nitric oxide (NO) production of Raw 264.7 mouse macrophage cells impaired by Nicotine. NO production was determined using Griess reagent assay. Cells were incubated with Nicotine (1 mM) and AFL (10, 50, 100, 200, 400 ug/mL) for 24 h. Results are represented as mean ± S.D. Normal : Not treated with Nicotine. Control : Treated with Nicotine only.
* represents $P < 0.05$ compared to the control.

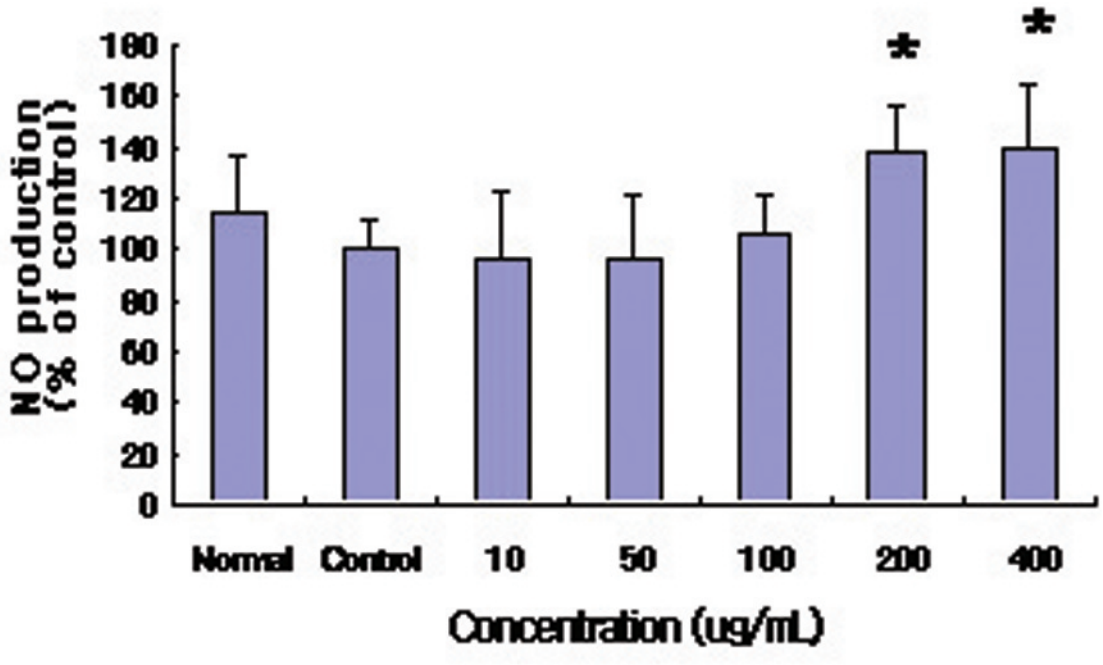

Fig. 4.: Effect of AFL on nitric oxide (NO) production of Raw 264.7 mouse macrophage cells impaired by Acetaminophen (AAP). NO production was determined using Griess reagent assay. Cells were incubated with AAP (2 mM) and AFL (10, 50, 100, 200, 400 ug/mL) for 24 h. Results are represented as mean ± S.D. Normal : Not treated with AAP. Control : Treated with AAP only.
* represents P< 0.05 compared to the control.

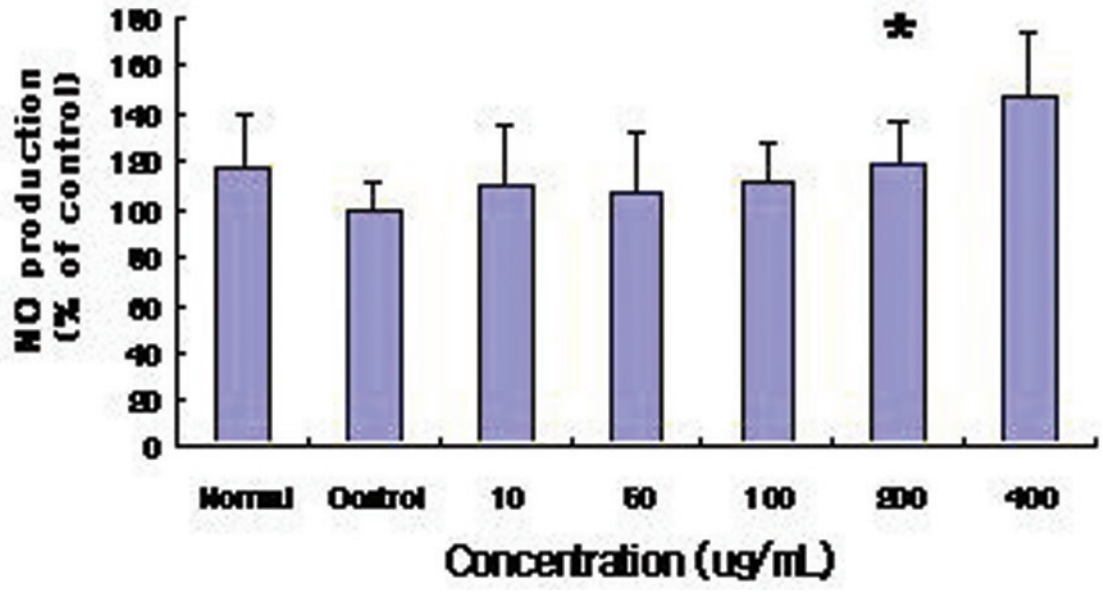

Fig. 5.: Effect of AFL on nitric oxide (NO) production of Raw 264.7 mouse macrophage cells impaired by Acetaldehyde (AC). NO production was determined using Griess reagent assay. Cells were incubated with AC (200 uM) and AFL (10, 50, 100, 200, 400 ug/mL) for 24 h. Results are represented as mean ± S.D. Normal : Not treated with AC. Control : Treated with AC only.
* represents P< 0.05 compared to the control.