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석사학위논문

질경이(*Plantago Asiatica*) 추출물이
인체 유방암 MDA-MB-231세포주에
미치는 영향

조선대학교 보건대학원

대체의학과

안 옥 녀

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on MDA-MB-231 Human Breast Cancer Cells

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지도교수 문 경 래

이 논문을 대체의학 석사학위신청 논문으로 제출함

2010년 10월

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ABSTRACT

Effects of *Plantago Asiatica* Extracts on *MDA-MB-231* Human Breast Cancer Cells

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Objective : This study was undertaken to evaluate the effects of the chemoprevention activity of *Plantago Asiatica* Extracts in MDA -MB-231 human breast cancer cell lines.

Methods : MTS assay was used to detect the effects of *Pantago Asiatica* Extracts on the MDA-MB-231 human breast cancer cell lines viability. Western Blotting was used to see the effects of *Plantago Asiatica* Extracts on cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression.

Results : Methanol Extracts of *Plantago Asiatica* Cell survival rate decrease depending on concentration, especially, it reduced about 80% cell survival rate at the log of 600 *ug/ml* shows the effect of suppression after treatment for 24 hours significantly. The expression of iNOS suppressed 200, 400*ug/ml* of concentration about 50%. But expression of COX-2 was not suppressed.

Hot water Extracts of *Pantago Asiatica* cell survival rate decrease depending on concentration. However, it reduced about 30% cell survival rate at the log of 900 *ug/ml* after treatment for 24 hours.

The expression of iNOS, COX-2, there was no significant effect of the inhibition

Conclusion : It can be concluded that *Plantago Asiatica* have an inhibitory effect on *MDA-MB-231* human breast cancer cells. So, It was expected that animal experiments and isolation and purification test of *Plantago Asiatica* would bring out more variety of clinical effects.

Key words: *Plantago Asiatica*, MDA-MB-231 human breast cancer cells, Cell survival rate, MTS assay, western blotting, COX-2, iNOS

I . INTRODUCTION

Since ancient times, breast is an organ of the body which is thought symbol of Aesthetic and maternal⁽¹⁾. October of each year is the month of breast cancer prevention all over the world. and put on the pink ribbon on the chest, there is the symbol for prevention consciousness improvement of breast cancer, beauty and health of female, that is symbol of freedom on breast.

Breast cancer occurs in the breast is a malignant cancer. Breast cancer is the most common cancer in women all over the world, especially the United States, Europe and developed countries women occur frequently. In particular, the United States 1 out of 7 women with breast cancer occurs, and 1 out of 33 women die from breast cancer incidence and mortality rates as high as seen from the disease are being investigated⁽¹⁾.

Breast cancer incidence is also increasing rapidly in Korea. Breast cancer incidence of Korea women's, it was 12.1 % in 1995, increased to 16.8 % in 2002. This trend of increasing incidence of breast cancer in the future be expected to be continued for a while.

It has been known that breast cancer is closely related to the influence of female hormones of estrogen but that has been reported, changes of lifestyle increase in incidence than estrogen. Increased in Obesity by dietary fat intake increase, decreased fertility and lactation,

Late Marriage, early menarche and late menopause, Such a social phenomenon, the incidence of breast cancer is predicted to increase.⁽²⁾

In current cancer treatments, surgical, radiation therapy, immunotherapy and chemotherapy has been used together. Because most of the drug as a chemically synthesized substances, they destroy malignant cells as well as even normal cells.

So, researches about natural products has been very carried out to develop breast cancer prevention and cancer-related adjuvant.⁽³⁾

The materials used in this study, plantain (*Plantago Asiatica*) is *Plantago* species belonging to *Plantaginaceae*. *Plantago Asiatica* is itself in Korea and China, Japan and East Asia and Central Asia. Strong vitality, fertility is excellent.

The stem is not original. The leaves are oval or egg shape. Length of 4~15cm width is 3~8cm in 5 parallel veins, wavy edge of the sawtooth. The flowers bloom from June to August in white, with a small funnel-shaped corolla is going out of a long pistil with multiple Stamens seems to ear.

From September to October is ripe seed, opened the lid comes out 6 to 8 black seeds. Ate the herb leaves and stems of young, hanging fruit juice mixed with oil, pepper and meat, and ate in the rubbing Plantain leaves and seeds have been used as herbal medicine.

The seeds of plantain has diuretic, brilliant, bright eyes and expectorant activity, relieve cough, and enhance liver function and stimulates the secretion of digestive juices and antibacterial and cholesterol-lowering effect that has been widely used in folk medicine at home since ancient times⁽³⁾.

Leaf of plantain contain idoid glycoside such as genipiosidic acid, aucubin, flavone glycoside such as acetoside, pantagoside, platagin honoplantagin ursolic acid and various other ingredients such as some sterol. Seed of platain contain mucilage, adenine, choline, and various fatty acids that have been known antibacterial, anti-inflammatory and anti-tumor effects⁽⁴⁾.

Recently, according to research report associated with *Plantago asiatica*, Jeong et.al suggests that Ethyl acetate fraction of plantain showed strong antibacterial activity in both gram-positive and gram-negative microorgarnism⁽⁵⁾.

Samuelsen in a review, Plantain contains ingredients that are biologically valid such as iridoid glycosides, polysaccharides, lipids, flavonoids terpenoids, alkaloids, organic acid.

The biological activity of the plantain extract was found wound healing activity. anti-inflammatory, analgesic, weak antibiotic antioxidant, immonomodulating and antiulcerogenic activity⁽⁶⁾. Huang et.al, reported that Acteoside, isoacteoside, polysaccharides ingredients from plantain

seeds had significant immounoenhancing activity significantly to induce maturation of dendritic cells⁽⁷⁾.

Choi et.al suggests that methanol Extracts of *Pantago Asiatica* had powerful glycation inhibitory activity, which are known to be included in the pathogenesis of aging-related complications and diabetes⁽⁸⁾. Tezuka et.al suggests that *Plantago Asiatica* showed significant inhibition in J774.1 macrophages of mouse peritoneal exudate⁽⁹⁾.

Chiang et.al reported that hot water extract of *Plantago Asiatica* possessed significantly inhibition activity on the proliferation of lymphoma (U937) and on viral infection (ADV-1and HSV-21) and carcinoma (cervix, kidney, bladder, bone, lung, stomach) cells⁽¹⁰⁾. Kim et.al elucidated that various iridoides of *Plantago Asiatica* seeds significantly inhibited COX-2⁽¹¹⁾.

Chung et.al reported that *Plantago Asiatica* essential oils (PAEO) inhibited 3-hydroxy-3-methyl-glutaryl-co-enzyme A reductase expression in vivo and in vitro and show to reduce choesterolaemic properoties in mice⁽¹²⁾. Fang et.al. suggested that Acteoside, isoacteoside of *Plantago asiatica* extract showed significant inhibitory activity of angiotensin converting enzyme(ACE)⁽¹³⁾. Kim reported that inhibitory effect of plantago asiatica Extracts on the growth of gastric and colon cancer cell lines more than 50%⁽¹⁴⁾.

Thus , plantain (*Plantago Asiatica*) with various effect, to investigate the effect on breast cancer cells (MDA-MB-231). In this study, *Plantago*

Asiatica was extracted with hot water and methanol for investigate the effect of human breast cancer cell lines (MDA-MB-231).

Cancer Cell growth inhibition of each extract was measured. The cell viability was measured by MTS assay. Expression level of COX-2 and iNOS in the cancer marker targets were measured by western blotting to determine ability to inhibit the expression of cancer targeting factors.

II. MATERIALS AND METHODS

1. Materials for experiments

The *Plantago Asiatica* were purchased in september 2010 from Wha-soon Herb medicine Cooperative for this study. *Plantago Asiatica* was dried up in shade with cutting slices and was grinded into powder. Two type Extracts was extracted from *Plantago Asiatica* powder for experiments. One is Hot Water Extract, the other is Methanol Extract.

2. Reagents

The first degree of methanol reagent was used in this study. Reagents used in this study run as follows; LPS, 3-4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium inner salt (MTS), 1% penicillin and streptomycin were purchased from Sigma Chem. Co. in the USA.

Dulbcco's Modified Eagle's Medium (DMEM) and 5% fetal bovine serum (FBS) were purchased from Gibco / Invitrogen (Grand Island, NY, USA) ELISA Kit was purchased from Pierce endogen (Rockford, IL, USA) Microplate reader (MolecularDevices, Sunnyvale, CA, USA)

3. Cell culture

Human breast cancer MDA-MB-231 Cell Lines were purchased from American Tissue Culture Collection (Manassas, VA).

Human breast cancer MDA-MB-231 Cell Lines were cultured in DMEM containing 5% fetal bovine serum (FBS) and 100 U/ml penicillin and 100 U/ml streptomycin in the atmosphere with 5% CO₂.

4. Preparation of *Plantago Asiatica*

Extracts of the powder of *Plantago Asiatica* (100g) was extracted during 3 hours with distilled-deionized hot-water (1.3 liter) and lyophilized. Subsequently solvent was removed. The obtained powder was melted by concentration and then filtered with a twofold filter paper (Whatman NO.1) for use.

Another way was that the powder of *Plantago Asiatica* (100g) was filtered during 48 hours after the methanol (1 liter) being poured. It was filtered out using a filter paper. Then it was extracted with evaporator which step was followed by cooling, lyophilization, and afterward got the reagent.

5. MTS assay

The effect of Extracts of *Plantago Asiatica* on cell viability were estimated according to the manufacturer's instructions of Cell Titer 96 Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI).

Human breast cancer MDA-MB-231 Cell Lines were seeded in a 96-well plate and then incubated with different time (24H, 48H) dependent concentrations of Extracts of *Plantago Asiatica* and cell viability were observed closely. MTS(3-(4,5-dimethylthiazol -20yl)-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium) solution quantified in an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 492 nm and 690 nm. The MDA-MB-231 cell lines survival rate were expressed with percentages.

6. Western blot analysis

Human breast cancer MDA-MB-231 Cell Lines after processing with Extracts of *Plantago Asiatica* were harvested and disrupted. The protein supernatant fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to membranes and blocked with 5% skim milk followed by hybridization with the indicated antibodies.

Protein bands with horse radish peroxidase-conjugated secondary antibody were observed closely with Chemiluminescence Detection Kit.

7. Statistical analysis

Data are given expression as mean value \pm standard deviation of three independent experiments accomplished in triplicate at least. Data were computed for statistical significance using Student's t-test. The minimum level of statistical significance was set at $p < 0.05$.

III. RESULTS

1. Influence on the survival rate of cells

1) methanol extract

To investigate the cytotoxicity of the methanol extracts from the *Plantago Asiatica* were used to treat MDA-MB-231 Cells with dependence on the concentration 200, 400, 600 $\mu\text{g/ml}$. After incubation of 24, 48 hours, the viability of the cells were measured by MTS assay.

As shown in Fig. 1, Cell survival rate decrease depending on concentration. It reduced about 40% cell survival at the log of 200 $\mu\text{g/ml}$, and it reduced about 60% cell survival at the log of 400 $\mu\text{g/ml}$. Especially, it reduced about 80% cell survival rate at the log of 600 $\mu\text{g/ml}$ shows the effect of suppression after treatment for 24 hours significantly.

After treatment for 48 hours, Cell survival rate decrease depending on concentration. It reduced about 40% cell survival rate at the log of 400 $\mu\text{g/ml}$, and it reduced about 60% cell survival rate at the log of 600 $\mu\text{g/ml}$,

The reduction of the viability of MDA-MB-231 cells was more effective in a short time, in a concentration dependent.

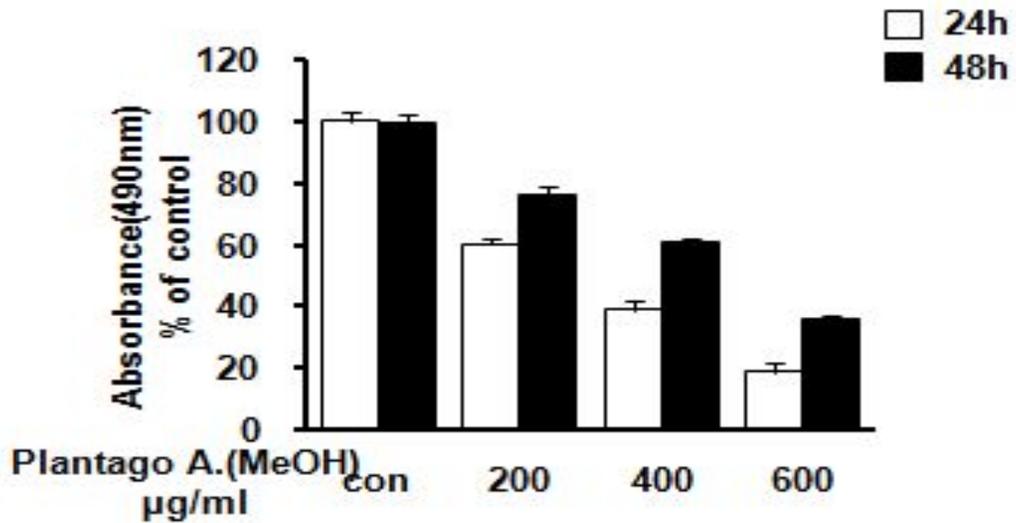


Fig. 1 Effects of *Plantago Asiatica* (methanol extracts) on the viability of MDA-MB-231 human breast cancer cells. The cells were incubated for 24, 48 hours after treatment.

2) Hot water extract

To investigate the cytotoxicity of the hot water extracts from the *Plantago Asiatica* were used to treat MDA-MB-231 Cells with dependence on the concentration 200, 400, 600 $\mu\text{g/ml}$. After incubation of 24, 48 hours, the viability of the cells were measured by MTS assay.

As shown Fig. 2, cell survival rate decrease depending on concentration. However, it reduced about 30% cell survival rate at the log of 900 $\mu\text{g/ml}$ after treatment for 24 hours.

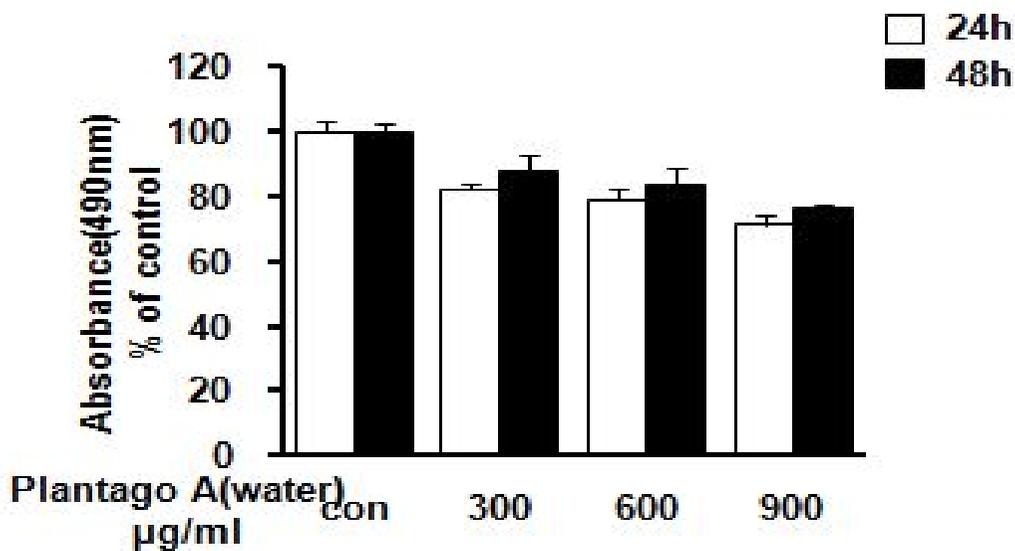


Fig. 2. Effects of *Plantago Asiatica* (hot water Extracts) on the viability of MDA-MB-231 human breast cancer cells. The cells were incubated for 24, 48 hours after treatment.

2. Influence of COX-2, iNOS expression

To investigate the COX-2, iNOS expression of the methanol and hot water extracts from the *Plantago Asiatica*, they were used to treat MDA-MB-231 Cells with dependence on the concentration 200, 400, 600 $\mu\text{g/ml}$. After incubation of 24 hours, expressions of COX-2, iNOS were compared after measuring β -actin by Western blotting.

As shown Fig.3, In methanol extracts, expression of iNOS suppressed 200, 400 $\mu\text{g/ml}$ of concentration about 50%. As shown Fig.4, In hot water extract, There was no significant effect of the inhibition.

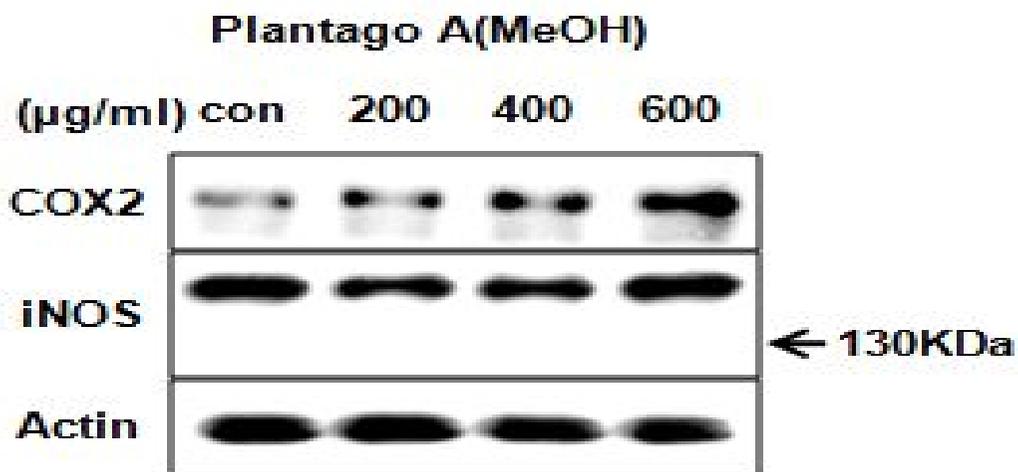


Fig. 3. Inhibitory effects of the concentration of methanol extracts from *Plantago Asiatica* against the COX-2 protein expression and iNOS expression of the MDA-MB-231 human breast cancer cells

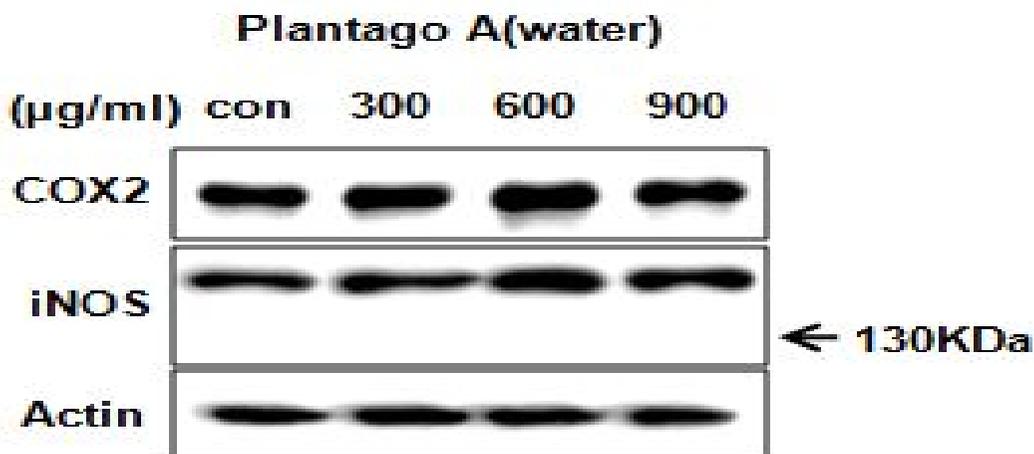


Fig. 4. Inhibitory effects of of the concentration of hot water extracts from *Plantago Asiatica* against the COX-2 protein expression and iNOS expression of the MDA-MB-231 human breast cancer cells

IV. DISCUSSION

Plantago asiatica is commonly used as folk herbal medicine in Korea, and other asian countries for treating infectious diseases associated with the urinary, respiratory, digestive tracts.

As the main bioactive components, Leaf of *Plantago Asiatica* contain iridoid glycoside such as geniposidic acid, aucubin, flavone glycoside such as acetoside, pantagoside, platagin honoplantagin ursolic acid and various other ingredients such as sterol kinds. Seed of platain contain mucilage, adenine, choline, and various fatty acids that have been known antibacterial, anti-inflammatory and anti-tumor effects.⁽³⁾

Anticancer research by using *Plantago Asiatica*, There are Study of apoptosis on gastric cancer⁽¹⁵⁾. and Study of cytotoxic, immunomodulatory effects on antileukemia, anticarcinoma (Bladder, bone, cervix, kidney, lung, and stomach)⁽¹⁶⁾.

Breast cancer is the most common malignancy among women in North America and Europe.⁽²⁾

In Korea, It has been increasing continuously over the years due to Westernization of diet, the reduction of fertility and breast-feeding and early menarche and late menopause, early detection of breast cancer⁽¹⁷⁾. In the breast cancer prevention research is focused on the development of prevention.

Hormonal regulators such as Tamoxifen reduced the incidence of invasive and non invasive breast cancer in high risk women⁽¹⁸⁾ but these drugs have side effects. In recent years, It has been very active prevention and drug development of breast cancer by using natural products.

Cyclooxygenase (COX, prostaglandine endoperoxide synthase) is a key enzyme for the prostanoids (prostaglandins, prostacyclins and thromboxanes) synthesis. There are two kinds of the isoform, one of them, COX 1 is expressed as components in most organizations and It is known to produce prostaglandine (PG) for general physiological function⁽¹⁹⁾.

The other one, COX-2 is a significant mediator of inflammation during both pathologic and physiologic responses to infectious agents and endogenous stimuli. Its overexpression has been detected several cancers including that of the breast, prostate, colorectal cancers. COX-2 inhibition suppress growth of tumor that was proved xenograft model in nude mouse⁽²⁰⁾.

Inhibition of COX-2 activity is associated that reduced Proliferation, migration, invasion, and matrix metalloproteinase (MMP) expression of breast cancer cell ⁽²¹⁾.

iNOS is the inducible form of NO. The cause of iNOS expression should occur necessarily in the process of removing by macrophage that inflammation caused by bacteria invading to the body. NO is a substance produced when the expression of iNOS. As a result, NO is a product

produced by the inflammation.

The expression of iNOS is produced excessive NO that inflammatory cytokine been released into the blood because of activation of immune cells caused by bacterial infections that is directly involved infiltration and invasion of bacterial infections.⁽²²⁾ The expression of iNOS in many different cancers have been reported ⁽²³⁾. In particular the activity of iNOS has been demonstrated in invasive breast cancer and INOS in the promotion of breast cancer tumors are known to response an important role.⁽²⁴⁾ NO is produced by iNOS activity of Breast cancer and stromal cell is known to increase tumor angiogenesis and tumor growth ⁽²⁵⁾.

In this study, *Plantago Asiatica* is known to have various effects in previous studies. *Plantago Asiatica* was extracted with methanol and hot water in order to verify effects in MDA-MB-231 human breast cancer cells. Cell viability was measured by MTS assay, COX-2 expression and i-NOS were measured by western blotting.

As a result, *Plantago Asiatica* methanol extract suppressed cell survival rate depending on concentration about 80% at 600 *ug/ml* and iNOS expression suppressed about 50%. These results seen that Methanol extract of the *Plantago Asiatica*, low molecular substance is expected to be effective in chemoprevention.

In addition, It was expected that animal experiments and isolation and purification test of *Plantago Asiatica* would bring out more variety of clinical effects.

V. CONCLUSION

To investigate the effect of *Plantago Asiatica* against human breast cancer, MDA-MB-231 cells. *Plantago Asiatica* was extracted with methanol and hot water in order to verify effects in MDA-MB-231 human breast cancer cells. Cell viability was measured by MTS assay. COX-2 expression and i-NOS was measured by western blotting. The results were as follows.

1. It was found that *Plantago Asiatica* methanol extract reduced the cell viability of MDA-MB-231 cells significantly. Treatment with 600 $\mu\text{g/ml}$ for 24 hours could result in about 80% inhibition. The reduction of the viability of MDA-MB-231 cells was more effective in a short time, in a concentration dependent (Fig 1).

2. *Plantago Asiatica* hot water extract reduced the cell viability of MDA-MB-231 cells. It reduced about 30% cell viability at the concentration 900 $\mu\text{g/ml}$ after treatment for 24 hours. (Fig.2).

3. It was observed that *Plantago Asiatica* methanol extract suppressed the iNOS expression at the concentration 200, 400 $\mu\text{g/ml}$ being compared after measuring β -actin by Western blotting. (Fig.3).

4. In hot water extract, there was no significant effect of the inhibition of expression of COX-2, iNOS. (Fig 4)

Therefore, by bioactivities, *Plantago Asiatica* inhibit the growth of cancer cells and *Plantago Asiatica* have the effect of suppressing promotion of breast cancer progression. So, *Plantago Asiatica* was thought to be effective on breast cancer

VI. KOREAN ABSTRACT

질경이 추출물이 인체유방암 세포주 MDA-MB-231에 미치는 영향

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목적 : 이 연구는 질경이 추출물이 인체 유방암 세포주인 MDA-MB-231에 항암 예방 활성 효과를 확인하기 위하여 이루어졌다.

방법 : 질경이 추출물의 인체 유방암 세포주인 MDA-MB-231 의 세포 생존 생존율은 MTS assay로 측정하였고 COX-2, iNOS 단백질 발현은 Western blotting으로 측정하였다.

결과 : 질경이의 메탄올 추출물은 인체 유방암 세포주인 MDA-MB-231 세포주의 암세포 생존율을 농도 의존적으로 감소시켰다. 특히 24시간 치료 후 농도 600 ug/ml에서 약 80%의 억제 효과를 나타냈다. iNOS의 발현은 농도 200, 400ug/ml 에서 약 50%의 억제 효과를 나타냈으며, COX-2 발현 억제 효과는 나타나지 않았다. 질경이의 열수 추출물에서는 24시간 치료 후 농도 900 ug/ml에서 약 30%의 세포 생존율 억제 효과를 나타냈으며, COX-2, iNOS의 발현 억제 효과는 나타나지 않았다.

결론 : 질경이 추출물은 인체 유방암 세포주인 MDA-MB-231의 세포 생존율 억제 효과가 있었으며 iNOS의 발현도 억제하였다. 향후 여러가지의 다양한 임상 효과를 확인하기 위하여 질경이의 정제 실험 및 동물 실험 등의 연구가 필요하다고 생각한다.

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저작물 이용 허락서

본인이 저작한 학위논문에 대하여 다음과 같은 방법 및 조건하에 대학교에 저작권을 위임할 것을 서약합니다.

1. 인터넷 및 온라인 서비스와 아카이빙을 위하여 저작물의 내용을 변경하지 않는 편집상 혹은 포맷상의 변경을 통한 복제를 허락함
2. 저작물의 DB 구축과 인터넷을 포함한 정보통신망에 공개하여 논문 일부 또는 전부의 복제·배포 및 전송을 허락함
3. 저작물에 대한 이용 기간은 3년으로 하고 계약 종료 2개월 이내에 별도의 의사표시가 없는 경우 기간을 계속 연장함
4. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판 허락을 하였을 경우 1개월 이내에 소속 대학에 통보함
5. 배포, 전송된 학위논문은 이용자가 다시 복제 및 전송할 수 없으며 이용자가 연구 목적이 아닌 상업적 용도로 사용하는 것을 금함
6. 소속대학은 학위논문 위임 서약 이후 해당 저작물로 인한 타인의 권리 침해에 관하여 일체의 법적 책임을 지지 않을 것을 확인함
7. 소속대학의 협약기관 및 한국교육학술정보원에 논문 제공을 허락함

동의여부 : 동의(✓) 조건부 동의() 반대()

※ 조건부 동의 및 반대인 경우 사유 및 조건을 기재하여 주시기 바랍니다.

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| 논문명(국문) | 질경이(Plantago Asiatica) 추출물이 인체유방암 MDA-MB-231세포주에 미치는 영향 | | |
| 논문주제분야 | 총류(), 철학(), 종교(), 사회과학(), 순수과학(✓), 해당란에 √표시 기술과학(), 예술(), 어학(), 문학(), 역사학() | | |
| 학위구분 | 석사 (✓) 박사 () | | 해당란에 √표시 |
| 초록기술언어 | 영 어, 한 글 | 논문 쪽수 | 34 쪽 |
| 한글초록 | <p>질경이의 메탄올 추출물은 인체유방암 세포주인 MDA-MB-231 세포주의 암세포 생존율을 농도 의존적으로 감소시켰다. 특히 24시간 치료 후 농도 600 ug/ml에서 약 80%의 억제효과를 나타냈다. iNOS의 발현은 농도 200, 400ug/ml 에서 약 50%의 억제효과를 나타냈고, COX-2, 발현억제효과는 나타나지 않았다.. 질경이의 열수 추출물에서는 24시간 치료 후 농도 900 ug/ml에서 약 30%의 세포 생존율 억제효과를 나타냈으며, COX-2, iNOS 발현억제효과는 나타나지 않았다.</p> <p>질경이 추출물은 인체 유방암 세포주인 MDA-MB-231의 세포생존율 억제효과가 있었으며 iNOS의 발현도 억제하였다. 향후 보다 다양한 임상효과를 확인하기 위하여 질경이의 정제 실험 및 동물 실험 등의 연구가 필요하다고 생각한다.</p> | | |
| 주제어(국문) | 질경이, 유방암세포주, 세포생존율 | | |
| 논문명(원문) | Effects of Plantago Asiatica Extracts on MDA-MB-231 Human Breast Cancer Cells | | |
| 본문기술언어 | 영 어 | | |
| 초록(원문) | <p>Methanol Extracts of <i>Plantago Asiatica</i> Cell survival rate decrease depending on concentration, especially, it reduced about 80% cell survival rate at the log of 600 ug/ml shows the effect of suppression after treatment for 24 hours significantly. The expression of iNOS suppressed 200, 400ug/ml of concentration about 50%. But expression of COX-2 was not suppressed. Hot water Extracts of <i>Pantago Asiatica</i> cell survival rate decrease depending on concentration. However, it reduced about 30% cell survival rate at the log of 900 ug/ml after treatment for 24 hours. The expression of iNOS, COX-2, there was no significant effect of the inhibition. So, it can be concluded that <i>Plantago Asiatica</i> have an inhibitory effect on <i>MDA-MB-231</i> human breast cancer cells. It was expected that animal experiments and isolation and purification test of <i>Plantago Asiatica</i> would bring out more variety of clinical effects.</p> | | |
| 주제어(원문) | <i>Plantago Asiatica</i> , MDA-MB-231 Cells, Cell survival rate | | |