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2011년 2월
석사학위논문

냉이(*Capsella bursa-pastoris*) 추출물이
인체 전립선암 세포주
(Human Prostate Cancer Cell Lines)에
미치는 영향

조선대학교 보건대학원

대체의학과

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ABSTRACT

Effects of *Capsella bursa-pastoris* Extracts on Human Prostate Cancer Cell Lines

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Objective : This study is on the effects of the inhibitory activity of *Capsella bursa-pastoris* extract in human prostate cancer cell lines.

Methods : MTS assay was used to detect the effects of *Capsella bursa-pastoris* extracts on human prostate cancer cell lines viability. Western Blotting was used to see the effects of *Capsella bursa-pastoris* extracts on COX-2 and iNOS expression.

Results : Methanol extracts of *Capsella bursa-pastoris* reduced human prostate cancer cell lines depending on time and concentration. Also they reduced COX-2 and iNOS according to concentration.

MTS assay results,

Methanol and hot water extracts result such as ;

1. Methanol extracts of *Capsella bursa-pastoris* reduced human prostate cancer cell lines depending on time.
2. Methanol extracts of *Capsella bursa-pastoris* reduced human prostate

cancer cell lines depending concentration.

Cell survival rate reduced depending on time and concentration especially, they reduced significantly 50% cell viability at 600 and 900 μ g/ml.

3. Hot water extracts of *Capsella bursa-pastoris* do not reduced human prostate cancer cell lines depending on time.
4. Hot water extracts of *Capsella bursa-pastoris* do not reduced human prostate cancer cell lines depending concentration.

Cell survival rate do not reduced depending on time and concentration.

Western blot results,

Methanol and hot water extracts result such as ;

5. Methanol extracts of *Capsella bursa-pastoris* reduced 80% expression of COX-2 in 600 and 900 μ g/ml and dramatically 95% expression of iNOS at 300 and 600 μ g/ml concentration.

6. Hot water extracts of *Capsella bursa-pastoris* reduced 50% expression of iNOS at 900 μ g/ml concentration.

Conclusion :

Methanol and hot water extracts result such as ;

Methanol extracts of *Capsella bursa-pastoris* reduced significantly 50% cell viability of human prostate cancer cell lines at 600 and 900 μ g/ml concentration and 80% expression of COX-2 in 600 and 900 μ g/ml and dramatically 95% expression of iNOS at 300 and 600 μ g/ml

concentration. Hot water extracts of *Capsella bursa-pastoris* reduced 50% expression of iNOS at 900 µg/ml concentration.

Methanol extracts of *Capsella bursa-pastoris* implied that it was expected that isolation and purification test bring out more various clinical effects.

Key words: *Capsella bursa-pastoris*, human prostate cancer cell lines, MTS, COX-2 and iNOS

I . INTRODUCTION

Prostate cancer is one of the most common cancers in American men. There are accounted about 232,000 new patients and about 30,000 men's prostate cancer death in 2008 in USA. About 1.3 million American men have been diagnosed and they are in pain with this cancer. This is because it affects elderly men, with an average age in the 70's or 80's.

According to the report of Korean national central cancer registration center in 2008, prostate cancer death is the first cause of men's cancer death in Korea.

Although researchers do not know the exact cause of prostate cancer in USA, they have identified several factors that increase the risk of getting this disease:

Older age – About 30% of men at age 60 have been diagnosed with prostate cancer. About 50% and 70% of men at age 80 are in pain with this cancer. The percent jumps to between men at age 60 and at age 80. This figure means about 3 out of 4 cases of prostate cancer in men over age 65.

Lifestyle factors – The risk of prostate cancer increases in obesity and eating a high-animal fat diet. Compared to men with normal body weights, obese men do not respond as well to treatment with prostate cancer as others.

Family history – In contrast with a man with no family history of the illness, the cancer risk of a man with family history jumps 2 to 3 times higher. When his father or brother had been diagnosed with this cancer, he must be more careful than a man with no family history.

The cancer is usually first suspected by an abnormal blood test, known as the prostate specific antigen (PSA) test. PSA is a protein made by the prostate and secreted into the semen. If prostate cancer spreads to the lymph nodes, bones or other organs, it can cause bone pain, weight loss, anemia, shortness of breath and other symptoms.

To observe prostate cancer, it can be examined a digital rectal exam to feel the prostate gland and checked lumps, hardness and enlargement in this exam.

At this time, the effective therapy for the prostate cancer cure is needed such as special chemoprevention. Apoptosis was induced in specific cancer cells to death automatically. Chemoprevention can be approved the treatment for prostate cancer cell decreation such as apoptosis. Chemotherapy is very effective in prostate cancer cure with using together other treatments such as a surgical operation and radiation therapy.¹⁾

In the recent research of prostate cancer cure, many medical plants are used and studied.²⁾ Many researches of other anticancer activity such as stomach cancer, breast cancer,³⁾ oral cancer, colon cancer,⁴⁾ liver cancer, etc were done about their anticancer effects.⁵⁾ But the studies of preventing prostate cancer have been not enough yet in medicinal and food plants such as *CBP*.⁶⁾

Further more *Capsella bursa-pastoris* are not verified still now about anticancer activity against prostate cancer. So this study is done with *Capsella bursa-pastoris* to know whether it can be used or not as chemoprevention and medical plants.

In this study, PC-3 human prostate cancer cell lines is tested on hot-water and methanol extracts of extracts of *Capsella bursa-pastoris*. This study will show two consequences, one is cell viability by MTS assay and the other is Cox-2 and iNOS expression by western blotting assay.

II. MATERIALS AND METHODS

1. Materials for experiments

Capsella bursa-pastoris used for the study was obtained from Cheon-An city in Korea. The whole plants were dried completely in the shade with cutting slices and then ground down to fine power.

2. Reagents

Methanol reagent in the first degree was used. 1% penicillin, and streptomycin were purchased from Sigma Company in the USA. Dulbeco's Modified Eagle's Medium(DMEM), containing 5% fetal bovine serum(FBS), (3-(4,5-dimethylthiazol-2-yl)-5-(3- carboxymethoxyphenyl) -2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS), ELISA microplate reader(Molecular Devices, Sunnyvale, CA, USA)

3. Cell culture

The PC-3 human prostate cancer cells were obtained from the American Tissue Culture Collection (ATCC)(Manassas, VA). The human prostate cancer cells were cultured in DMEM containing 5% fetal bovine serum(FBS) and 100 U/ml each of penicillin and streptomycin in an atmosphere containing 5% CO₂.

4. Preparation of *Capsella bursa-pastoris*

Hydrothermal extract was drawn out through these processes. The first process was that 1.3 liters of the distilled water was added to the dried *Capsella bursa-pastoris*(100g). Then it was heated in a 1.3 liter

sized earthen pot preparing a medicinal decoction (DWP-5000 M) for 3 hours and extracted.

After centrifugal separation of the output, only the upper layer was given a decompression filtration through a twofold filter paper(Whatman NO.1). One liter of methyl alcohol was poured into the dried total *Houttuynia cordata* Tunb(100g). After 24 hours, the impurities were filtered out using a filter paper. The sample was obtained by hot-water and lyophilization.

5. MTS cytotoxicity

The effect of extracts of *Capsella bursa-pastoris* on viability was estimated using the Cell Titer 96 AQueous. One Solution Cell Proliferation Assy Kit (Promega, Madison, WI) according to the manufacturer's instructions. Cells were seeded in a 96-well plate and then incubated with different time dependent concentrations of extracts of *Capsella bursa-pastoris* MTS solution quantified in an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 492 nm and 690 nm background.

6. Western blot analysis

After treating with extracts of *Capsella bursa-pastoris*, cells were harvested and disrupted. The protein supernatant fractions were subjected to SDS-PAGE(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 the horse radish peroxidase-conjugated secondary antibody were observed with a chemiluminescence detection kit.

7. Statistical analysis

Data are represented as mean \pm S.D. of at least 3 independent experiments performed in triplicate. Data were assessed for statistical significance using Student's t-test. The minimum level of significance was set at $p < 0.05$.

III. RESULTS

1. Influence on the survival rate of cells

To identify if the extracts from *Capsella bursa-pastoris* have cytotoxicity against the human prostate cancer cell lines, an MTS assay was conducted after treatment for 24 and 48 hours according to concentration.

1) Effects of *Capsella bursa-pastoris* extracts on the human prostate cancer cell lines viability using MTS assay (Methanol extracts)

To look into the cytotoxicity of the extracts from *Capsella bursa-pastoris* were used to treat the human prostate cancer cell lines with dependence on the concentration (0, 300, 600, 900 $\mu\text{g}/\text{ml}$). After 24 and 48 hours, the survival rate of the cells were measured.

In Figure 1, the effect of suppression as a result of treatment for 48 hours was shown about 40% at the log of 600 $\mu\text{g}/\text{ml}$ and about more than 50% suppression at the log of 900 $\mu\text{g}/\text{ml}$. It can be stated that the methanol extract from *Capsella bursa-pastoris* are effective depending on concentration.

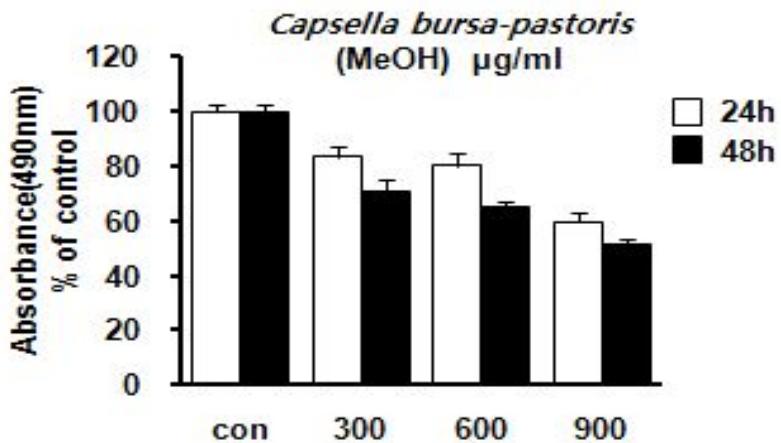


Figure 1. Effects of methanol extracts of *Capsella bursa-pastoris* on the anticancer activity of human prostate cancer cell lines of 24 and 48 hours after treatment with dependence on the concentration (0, 300, 600, 900 $\mu\text{g/ml}$).

2) Effects of *Capsella bursa-pastoris* extracts on the human prostate cancer cell lines viability using MTS assay (Hot water extracts)

To look into the cytotoxicity of the extracts from *Capsella bursa-pastoris* were used to treat human prostate cancer cell lines with dependence on the concentration (0, 300, 600, 900 $\mu\text{g/ml}$).

After 24 and 48 hours, the survival rate of the cells were measured. However, there was no effect as a result of the experiment. (Figure 2)

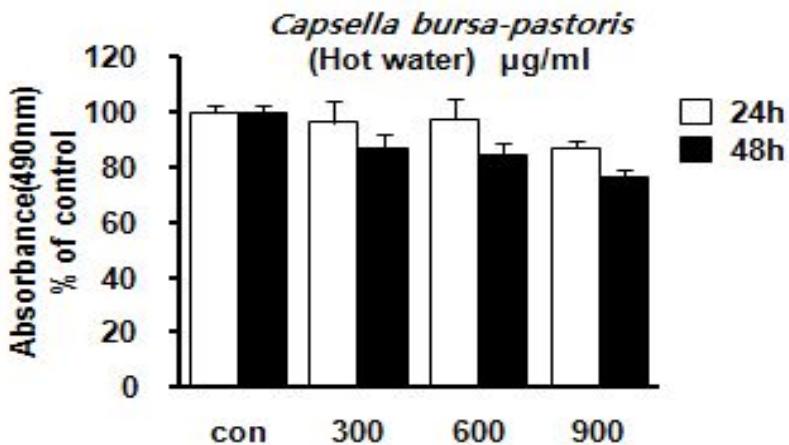


Figure 2. Effects of hot water extracts of *Capsella bursa-pastoris* on the anticancer activity of human prostate cancer cell lines of 24 and 48 hours after treatment with dependence on the concentration (0, 300, 600, 900 $\mu\text{g/ml}$).

2. Western blotting

1) Effects of methanol extracts of *Capsella bursa-pastoris* on cyclooxygenase-2 (COX-2) and Inducible Nitric oxide synthase (iNOS) expression in human prostate cancer cell lines by Western Blotting.

In the control group and the test groups, Cyclooxygenase-2 (COX-2) and Inducible Nitric oxide synthase (iNOS) expressions were compared after measuring β -actin to verify equal loading of protein in each lane.

In the test groups. At the log of 600 and 900 $\mu\text{g/ml}$, the suppression of the expression of COX-2 is observed as 85%. And at the log of 300

and 600 $\mu\text{g/ml}$, the suppression rate of the expression of iNOS is observed dramatically as 95%. (Figure 3)

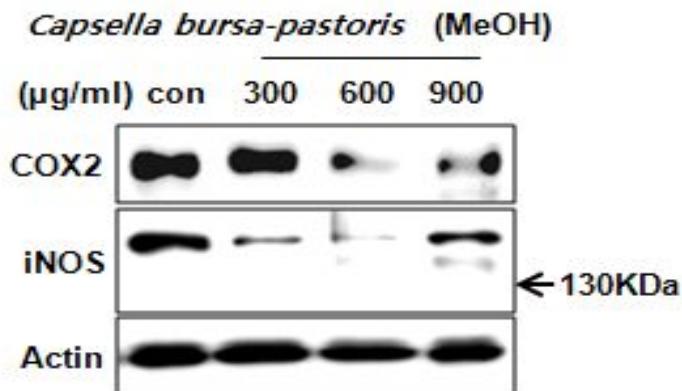


Figure 3. Inhibitory effects of classified methanol extracts of *Capsella bursa-pastoris* on the COX-2 and iNOS expression of human prostate cancer cell lines.

2) Effects of hot water extracts of *Capsella bursa-pastoris* on cyclooxygenase-2 (COX-2) and Inducible Nitric oxide synthase (iNOS) expression in human prostate cancer cell lines by Western Blotting.

In the control group and the test groups, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions were compared after measuring β -actin to verify equal loading of protein in each lane. In the test groups, The suppression of the expression of COX-2 and iNOS is NOT observed. (Figure 4)

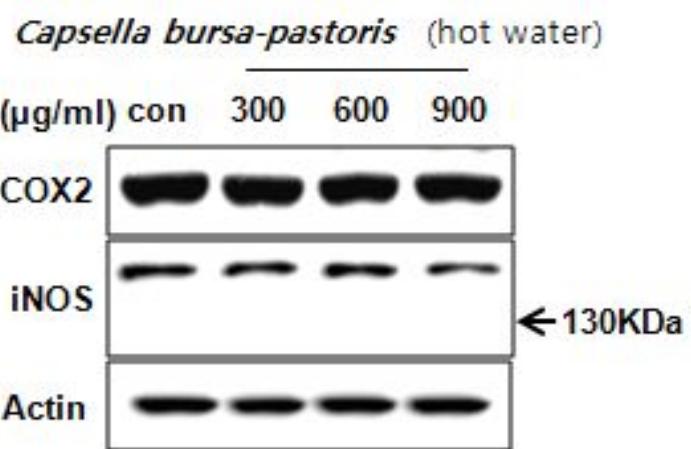


Figure 4. Inhibitory effects of classified hot water extracts of *Capsella bursa-pastoris* on the COX-2 and iNOS expression of human prostate cancer cell lines.

IV. DISCUSSION

Capsella bursa-pastoris, known by its common name shepherd's-purse because of its triangular, purse-like pods, is a small (up to 0.5m) annual and ruderal species, and a member of the Brassicaceae or mustard family.⁷⁾ It is native to eastern Europe and Asia minor⁸⁾ but is naturalized and considered a common weed in many parts of the world, especially in colder climates, including Britain, where it is regarded as an archaeophyte, North America and China, but also in the Mediterranean and North Africa.

Capsella bursa-pastoris is gathered from the wild or grown for food to supplement animal feed, for cosmetics, and for medicinal purposes.⁹⁾ It is commonly used as food in Korea and China. It is one of the ingredients of the symbolic dish consumed in the Japanese spring-time festival.

Capsella bursa-pastoris is one of very important drugplants. When dried and infused, it yields a tea which is still considered by herbalists one of the best specifics for stopping haemorrhages of all kinds - of the stomach, the lungs, or the uterus, and more especially bleeding from the kidneys.¹⁰⁾

Herbally, it is primarily used to stop vaginal bleeding, an action which may be attributable to the common parasitic fungus found with it, which is related to the vasoconstrictor ergot.

Capsella bursa-pastoris contains fumaric acid as one active substance that can be isolated.¹¹⁾ Although fumaric acid and its derivatives are used with success in many conditions, there is no direct evidence that plant extract has been used with similar success.¹²⁾

Fumaric acid esters are used to treat psoriasis,¹³⁾ as it has been suggested that the condition is caused by an impairment of fumaric acid production in the skin.¹⁴⁾ Basically, esters are formed by condensing an acid with an alcohol. A fumaric acid ester is currently under investigation for treatment of multiple sclerosis.¹⁵⁾

In vitro studies with the ester, dimethyl fumarate (DMF) described an suppressive effect on nuclear factor kappa B (NF- κ B)-dependent transcription of tumor necrosis factor-alpha (TNF- α) induced genes in human endothelial cells.¹⁶⁾

The mice treatment with *Capsella bursa-pastoris* herb extract injections (0.14 g/kg/day) caused 80% restraint of Ehrlich tumor cells growth. The treated mice's tumor lumps showed varifocal necroses.¹⁷⁾

Experiments were also performed to isolate and identify the active component for the antitumor action, and an acidic substance was isolated in crystalline form from the herb extract. This acidic substance was identified as fumaric acid and was effective in inhibiting the growth of Ehrlich solid tumor at a dose of 10 mg/kg/day. The 50% lethal dose (i.p.) of this acid was 266 mg/kg.¹⁸⁾

The purpose of this research is to investigate whether the two type

extracts of *Capsella bursa-pastoris* (*CBP*) can reduce biomarker of cancer or not¹⁹⁾ and observe whether methanol and hot water extract of *CBP* can restrain cell viability or not.

Through the above result, *Capsella bursa-pastoris* (*CBP*) is conformed very wonderful medicinal plants for preventing prostate cancer because of surprising suppression rate of 95% iNOS expression on the low density of 600 $\mu\text{g/ml}$.²⁰⁾

If extracts of *Capsella bursa-pastoris* will be isolated and purified,²¹⁾ the higher inhibiting effects could be expected on the prostate cancer.²²⁾ And the research on activating substance of *CBP* should go on for chemoprevention²³⁾ against prostate cancer.²⁴⁾

V. CONCLUSION

The main purpose of this research is to investigate whether the two type extracts of *Capsella bursa-pastoris* (*CBP*) can reduce biomarker of cancer such as the COX-2 and iNOS expression of human prostate cancer cell lines or not.

The other purpose of this research is to observe whether methanol and hot water extract of *CBP* can restrain cell viability depending on time and concentration or not.

From the above result, *Capsella bursa-pastoris* (*CBP*) is thought very useful medicinal plants for prostate cancer because of surprising suppression rate of 95% iNOS expression. Making still more wonderful, 95% suppression rate was observed dramatically on the low density of 600 $\mu\text{g/ml}$.

The higher anticancer effects can be expected on the isolated and purified extract of *Capsella bursa-pastoris*. And the research on activating substance of *CBP* should go on.

1. In methanol extracts, Cytotoxicity for the human prostate cancer cell lines depends on the density of methanol extracts. In 48 hours, especially, it resulted in an 50% growth obstruction rate in 900 $\mu\text{g/ml}$.
2. In hot water extracts of *Capsella bursa-pastoris* cases, cytotoxicity for the human prostate cancer cell lines has no reaction of anticancer

restraints effect.

3. In methanol extracts, COX-2 expression is restrained 80% in 600 and 900 $\mu\text{g/ml}$ modulation and iNOS expression is restrained 95% in 300 and 600 $\mu\text{g/ml}$ modulation.

4. In hot water extracts, COX-2's and iNOS expression is not restrained.

VI. KOREAN ABSTRACT

냉이 추출물이 인간 전립선암 세포주에 미치는 영향

지도교수 : 문 경 래

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대체의학과 진 정 원

본 논문의 목적은 식용식품인 냉이를 열수와 메탄올로 추출하여 시간과 농도를 달리하여 저분자량과 고분자량 상태에서의 인체 전립선암 세포주에 대한 세포 생존율과 암 표적인자 발현을 조사하여 천연추출물의 항암예방 가능성을 알고자 했다.

본 연구에서 두 가지 실험 방법 즉 MTS assay 와 Western Blot assay를 사용하여, 냉이의 열수 및 메탄올 추출물이 인체 전립선암 세포주의 세포생존을 얼마나 억제하는지를 측정하기 위하여 MTS assay를 시행했고, 냉이의 두 가지 추출물이 암 표적인자 COX-2와 iNOS의 발현 억제능을 측정하기 위하여 Western Blot assay를 시행하였다.

실험 결과 농도 300 $\mu\text{g}/\text{ml}$ 과 600 $\mu\text{g}/\text{ml}$ 의 냉이 메탄올 추출물에서 암 표적인자 iNOS 발현량이 놀랍게도 95%나 억제되었고, 또 다른 암 표적인자인 COX-2 발현량은 냉이 메탄올 추출물 600 $\mu\text{g}/\text{ml}$ 과 900 $\mu\text{g}/\text{ml}$ 의 농도에서 95%나 억제되었다. 또한 열수 추출물에서는 농도 900 $\mu\text{g}/\text{ml}$ 에서 암 표적인자 iNOS 발현량이 50% 억제되었다. 세포생존율 억제는 600 $\mu\text{g}/\text{ml}$ 과 900 $\mu\text{g}/\text{ml}$ 농도의 냉이 메탄올 추출물에서 50%의 억제능을 보였다.

그래서 본 연구의 결론은 냉이는 메탄올추출물 저농도 상태에서 암 표적인

자 iNOS 발현량의 놀라운 억제능을 보였고, 시간과 농도에 따른 암세포 생존율 억제능을 보였다. 그러나 열수 추출물에서는 유의적 억제 효과가 낮게 나타남으로서, 2000 μ g/ml 내지 3000 μ g/ml 수준의 고농도에서의 추가적 실험과 함께 메탄올 추출물에 대한 IN VIVO 실험이 요구된다.

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저작물이용허락서

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(V) 조건부 동의() 반대()

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

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논문명(국문)	냉이 추출물이 인체전립선암 세포주에 미치는 영향		
논문주제분야	종류(), 철학(), 종교(), 사회과학(), 순수과학(), 기술과학(<input checked="" type="checkbox"/>), 예술(), 어학(), 문학(), 역사학()		
학위구분	석사 (<input checked="" type="checkbox"/>) 박사 ()		
초록기술언어	영어, 한글	논문 쪽수	32 쪽
한글초록	<p>실험 결과 농도 300$\mu\text{g}/\text{ml}$ 과 600$\mu\text{g}/\text{ml}$의 냉이 메탄올 추출물에서 암 표적인자 iNOS 발현량이 놀랍게도 95%나 억제되었고, 또 다른 암 표적인자인 COX-2 발현량은 냉이 메탄올 추출물 600$\mu\text{g}/\text{ml}$ 과 900 $\mu\text{g}/\text{ml}$의 농도에서 95%나 억제되었다. 또한 열수 추출물에서는 농도 900$\mu\text{g}/\text{ml}$에서 암 표적인자 iNOS 발현량이 50% 억제되었다.</p> <p>세포생존율 억제는 600$\mu\text{g}/\text{ml}$ 과 900$\mu\text{g}/\text{ml}$ 농도의 냉이 메탄올 추출물에서 50%의 억제능을 보였다. 송이버섯 메탄올 추출물은 농도가 높아짐에 따라 (900 $\mu\text{g}/\text{ml}$) 암 표적인자 iNOS 발현량을 놀랍게도 80%나 억제시켰고, COX-2 발현량도 40% 감소시켰다. 또한 시간이 경과함에 따라 48시간 만에 농도 900 $\mu\text{g}/\text{ml}$에서 암 세포 생존율을 65%나 억제시켰다.</p>		
주제어(국문)	냉이, 전립선암, 세포생존율		
논문명(원문)	Effects of <i>Capsella bursa-pastoris</i> Extracts on Human Prostate Cancer Cell Lines		
본문기술언어	영어		
초록(원문)	Methanol extracts of <i>Capsella bursa-pastoris</i> reduced significantly 50% cell viability of human prostate cancer cell lines at 600 and 900 $\mu\text{g}/\text{ml}$ concentration and 80% expression of COX-2 in 600 and 900 $\mu\text{g}/\text{ml}$ and dramatically 95% expression of iNOS at 300 and 600 $\mu\text{g}/\text{ml}$ concentration. Hot water extracts of <i>Capsella bursa-pastoris</i> reduced 50% expression of iNOS at 900 $\mu\text{g}/\text{ml}$ concentration.		
주제어(원문)	<i>Capsella bursa-pastoris</i> , human prostate cancer cell lines, iNOS		