참깨품종의 리그난(lignan)성분 조성과 참깨종자로부터 추출한 세사미놀(sesaminol) 성분의 항종양효과

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Lignan Compositions of Sesame Varieties and Antitumor Activity of the Crude Sesaminol obtained from Sesame Seed

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실 험 목 적

참깨 주요유전자원의 리그난물질조성을 구명하여 고함유 리그난 품종육성의 육종자료로 활용함과 동시에 참깨의 부가가치증대로 새로운 수요창출에 목적이 있음.

재료 및 방법

- 공시재료 ; PI200100등 25종
- 추출, 분리 및 동정;

  n-hexane 탈지, 80% ethanol 추출, Prep. HPLC, ^{1}H-NMR, ^{13}C-NMR

o 정량분석 ; HPLC(Gulliver Jasco DG-980), 80% ethanol (Develosil ODS-5 column)

HPLC condition : Linear gradient 30% MeOH→80% MeOH, Flow rate ; 1.0 ml/min

o Antitumor실험

재료; (^{3}H)Thymidine(50 μCi/μmol),(^{3}H)uridine(55 μCi/μmol),(^{3}H)leucine(150 μCi/μmol)-Du Pont Chemical Company; HL-60 Cells (American type cell cultures), RPMI medium, fetal calf serum, trypsin, penicillin and streptomycin-GIBCO Company; Diaion HP 20-Mitsubishi Chemical Company (Japan)

HL-60 Cell 배양: 100mm배양접시(10% fetal calf serum + 1% penicillin-streptomycin, 5% CO_2, 37° C)-4일간, HL-60 Cell 안에서 DNA, RNA와 단백질합성의 측정

결과 및 고찰

1. 참깨 종실의 리그난성분(sesamin, sesamolin, sesaminol tri, di and monoglucoside, sesamolinol)을 동시정량할수 있는 기술을 개발하였다.

2. 참깨종실에서 리그난배당체의 함량은 sesaminol triglucoside>sesaminol diglucoside >sesaminol monoglucoside 순이었으며, 25품종의 분석결과 100g 종자에는 평균 sesamin 380.6mg(18.5-820.5), sesamolin 276.5mg(20.3-680.9), sesaminol triglucoside 68.4mg (14.1-91.3), sesaminol diglucoside 11.6mg(8.2-18.3), sesaminol monoglucoside 8.3mg (5.4-19.5), sesamolinol 20.5mg(5.6-28.5)의 함량(변이폭)이었다.

3. 참깨종자로부터 추출한 sesaminol성분은 항종양활성(사람백혈병, HL-60 Cell)을 가지고있으며, 60-100μg/ml 함량범위에서 HL-60세포생장억제, 200μg/ml이상에서 세포상해를 보여주었다.

4. Sesaminol성분함량이 60μg/ml 수준에서 HL-60 Cell의 DNA, RNA, 단백질합성의 억제정도는 각각 83%, 76%, 60%이었다

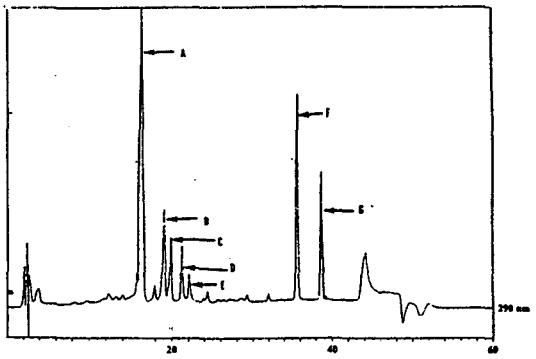

Fig 3 High performance liquid chromatogram of sesaminol glucosides, sesamolinol, sesamin and sesamolin components extracted from sesame seed of P1200100 sesame variety.
Peak A, sesaminol triglucoside : B, sesamolinol(1) : C, sesaminol diglucoside : D,sesamolinol(2) : E, sesaminol monoglucoside : F, sesamin : G, sesamolin.

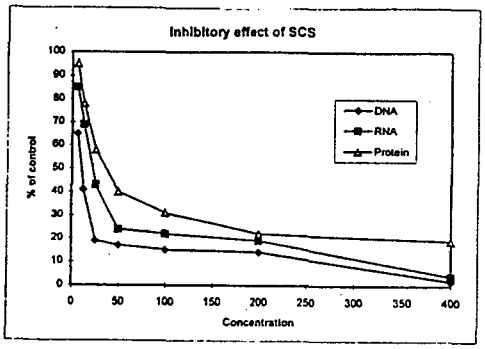

Fig.5. Inhibitory effect of various concentration of SCS on the synthesis of DNA, RNA and protein in HL-60 cells. The SCS was added, at the indicated final concentration, to 1.0 ml of HL-60 cells, suspended in RPMI medium without calf serum at concentration of 5 x 10^5 cells/ml. [^{3}H]Thymidine (50 μ Ci/ μmol : 3 μl), [^{3}H]uridine (55 μCi/ μmol : 5 μl) or [^{3}H]leucine (200 μCi/ μ mol :10 μl) were added. The cells were incubated at 37°C for 120 min, the reactions were terminated by addition 2 ml of cold PBS and the rate of DNA, RNA and protein synthesis were determined as described in section 2. The percentage of incorporation shown is expressed relative to cell cultures to which no inhibitor was added.

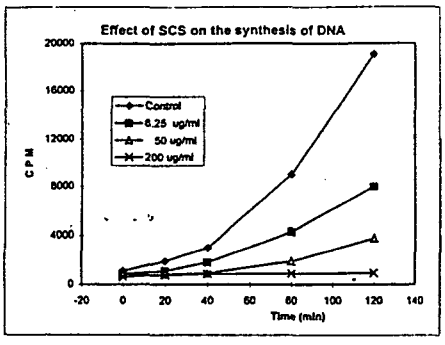

Fig.6. Effect of SCS on the synthesis of DNA in HL-60 cells at various times following exposure to inhibitor. HL-60 cells (5 x 10^5 cells/ml), suspended in RPMI medium, were divided into 4 portions. SCS was added to each of 3 portions of cultures at a final concentrations of 6.25, 50 or 200 μg/ml. DMSO was added to one of the 4 portions of cultures and served as positive control. [^{3}H]thymidine (50 μCi/ μmol : 3 μl) was added to each portion. The cultures were further incubated at 37°C, and at the indicated times 1 ml of sample was pipetted into 2ml of cold PBS and the rate of DNA synthesis were determined as described in Section 2.

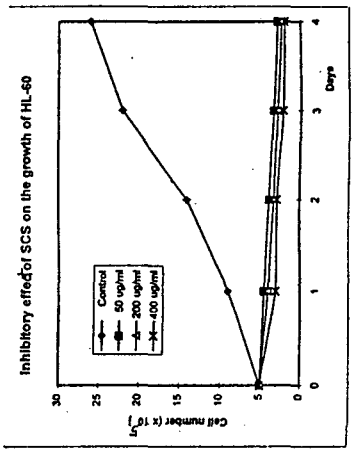

Fig.4 Inhibitory effect of various concentrations of SCS on the growth of HL-60 cells. HL-60 cells (5 x 10^5 cells/ml), suspended in RPMI medium supplemented with 10% calf serum and 1% penicillin and streptomycin, were incubated with various concentration of SCS and control (vehicle only) at 37°C for 4 days. Every 24 h, the number of HL-60 cells was counted under a microscope as described in Section 2.

Table 3 Irreversible inhibitory effect of the SCS on the synthesis of DNA in HL-60 cells

Concentration of SCS (μg / ml)	[^{3}H]Thymidine incorporation into TCA insoluble materials		% of control	
	A	B	A	B
0	14077	15555	100	100
12.5	7320	7778	52	50
50	2112	933	15	6
200	563	466	4	3

A, HL-60 cells (5 x10^5 cells/ml) were preincubated with 2 μl DMSO, or SCS in DMSO at 37°C for 120 min, then cells were washed with PBS 3 times, each time with 2 ml of PBS to remove the SCS, Cells were resuspended in fresh RPMI medium and 2 μl DMSO and [^{3}H]thymidine was added, incubated at 37°C for 120 min. The radioactivity incorporation into TCA insoluble materials was determined as described in Section 2. B, HL-60 cells (5 x10^5 cells/ml) were incubated with 2 μl DMSO at 37°C for 120 min, then cells were washed with PBS 3 times, each time with 2 ml of PBS to remove the DMSO, Cells were resuspended in fresh medium and 2 μl DMSO or SCS in DMSO and [^{3}H]thymidine was added, incubated at 37°C for 120 min. The radioactivity incorporation into TCA insoluble materials was determined as described in Section 2.