

1 . 1 . 1 . 2 . 1

1
2

I. 가 , Flubiprofen, Ibuprofen
NSAID

prostaglandin, IL - 1 가 , 가

1).

8 - 12). 가

2, 3).

가

가

8),

13 - 20)

PDGF - BB, IGF - 1, TGF -

4 - 6).

Sanguinaria

21)

가

tetracycline, penicillin 7), phe -
nolic compound, bisbiguanide(chlorhexi -
dine)

PGE₂

가 22 - 26).

(紅花, Safflower : Carthamus tincto -
rius Linne 27, 28), 29)

stasis) (blood 60 3 2 , .
가 (140g) .

(Magnoliae cortex) magnolol honokiol , (Zea Mays L)

가 , 1 : 1, 1 : 2, 1 : 5, 1 : 10
(lipid peroxidation) 100μg
- (tocopherol) /Ml .
1000 가 ,
가

가
30 - 32).

Streptococcus mutans

가 ,

가 , IL - 1

PGE₂ 22 -
26).

가 .

II.

1.

(Magnoliae cortex)
70% (EtOH) 3 2

(紅花, Safflower : Carthamus tinctorius
Linne) 300g 70%

2.

(1) 4×10^4 cells/cm²

35mm 10% FBS 1%

antibiotic - antimycotic solution -

MEM 37 °C 100%

1 95% 5% CO₂

1/3 3. 가

35mm 1%

antibiotic - antimycotic solution (Gibco, Grand Island, NY, USA) 10% fetal bovine serum (FBS; Gibco) 가 - minimum essential medium (MEM; Gibco)

0.25% trypsin - EDTA

가 well 1×10^5

96 well plate (Nunc, USA) . 24

HBSS

Rapid colorimetric

95% 95%, 5% CO₂ 37

MTT assay³⁴⁾ 가

가 200 μ l 가 24

(2)

Bellow 33)

21

가

0.1% collagenase (Sigma, St Louis, MO, USA) 4mM EDTA

0.25% trypsin (Gibco)

1.5 ml 37 °C 가

Reacti - vial (Pierce, Rockford, IL, USA) 10,

20, 30, 50 70

ice - cold FBS

50 70

400 x g 10

Hank's balanced salt solution (HBSS; Gibco)

methyl thiazol - 2 - YL - 2, 5 - diphenyl tetrazolium bromide (MTT) (Sigma) 50 μ l well , 4

MTT formazon dimethyl sulfoxide (Sigma) 50 μ l 가

Plate Spectrophotometer (Thermo max, molecular devices, Melano - Park, CA, USA) 570nm

4. Alkaline phosphatase (ALPase)

96 well culture plate well 1×10^4

, 24 , 가

48
 1% Triton X - 100
 , sonication . 0.4mM Tris HCl,
 2mM MgCl₂, 4mM p - nitrophenol phosphate
 가 50μℓ/well 가 30
 , 150μℓ/ 1N NaOH 가
 p - nitro phenol
 405nm spectrophotometer
 . ALPase

5. 가
 , 24
 multi - well dish well 1 × 10⁴ cell
 , 3
 HBSS , 10%
 FBS, 10 units/Mℓ streptomycin, 0.2μℓ/Mℓ
 formazone - MEM 0.9Mℓ
 0.1Mℓ sample , 22 [³H] -
 thymidine well 5 μCi 가 2
 ice - cold 5% TCA(Trichloroacetic acid) 3Mℓ
 4 10 TCA 3 - 4
 0.5N NaOH
 1Mℓ 37 30
 100μℓ cocktail solution 4Mℓ 가
 liquid scintillating counter(Beckman,
 CA, USA) count per minute

6.

(1)

가 1 : 5, 1 : 10
 0.25g/kg , 0.5g/kg
 0.1g/kg ,

0.5g/kg

1 2 oral zonde needle

(2)

200 - 250g 30mg/kg 2%
 (Rompun ,
 (Ketalar , ,)

0.5% chlorhexidine

(cephalostat)

8mm trephine bur(3i, FL, USA)

8mm layered
 suture 4

10% formalin , 10% nitric acid

5 paraffin
 6μm

Masson - trichrome

(Olympus BH - 2,
 Olympus Co., Tokyo, Japan)

digital color
 camera(SDC - 4304PF, Samsung Co., Seoul,
 Korea)

(Bildanalyssystem AB,
 Sweden)

7.

Table 1. Effects of safflower extracts and Magnoliae cortex extract on the growth and survival of human periodontal ligament cells(PDL cells) and fetal rat calvarial osteoblasts(by rapid col -

Groups	Growth and survival rate(%) ^a		
	Mixture ratio (MCE : SSE)	Osteoblast	PDL cell
MCE(100µg/Mℓ)		126.2	110.9
SSE(100µg/Mℓ)		118.1	112.3
ZML(100µg/Mℓ)		118.8	102.7
MSM(100µg/Mℓ)	1 : 1	124.9	143.8
MSM(100µg/Mℓ)	1 : 2	139.3	155.1
MSM(100µg/Mℓ)	1 : 5	163.4	175.3
MSM(100µg/Mℓ)	1 : 10	183.2	173.9

MCE : Magnoliae cortex extract, SSE : Safflower seed extract, ZML : Zea May L extract, MSM: Magnoliae cortex and Safflower seed extract mixture, ^a(mean O.D. of each test group / mean O.D. of control group) ×

Table 2. Effects of safflower extracts and Magnoliae cortex extract on the ALPase activities of human periodontal ligament cells(PDL cells) and fetal rat calvarial osteoblasts

Groups	Mixture ratio (MCE : SSE)	ALPase activity(nM/30min/mg protein) ^a	
		Osteoblast	PDL cell
control		234.5 ± 19.4	224.5 ± 21.5
MCE(100µg/Mℓ)		242.1 ± 15.8	233.1 ± 20.2
SSE(100µg/Mℓ)		256.7 ± 21.7	247.4 ± 18.1
ZML(100µg/Mℓ)		248.3 ± 23.4	242.3 ± 17.7
MSM(100µg/Mℓ)	1 : 1	275.9 ± 16.4*	271.8 ± 14.4*†
MSM(100µg/Mℓ)	1 : 2	271.6 ± 15.1*	267.2 ± 16.7*†
MSM(100µg/Mℓ)	1 : 5	304.8 ± 15.7*†‡	298.5 ± 15.3*†‡
MSM(100µg/Mℓ)	1 : 10	352.1 ± 18.3*†‡	312.6 ± 16.6*†‡

MCE : Magnoliae cortex extract, SSE : Safflower seed extract, ZML : Zea May L extract, MSM: Magnoliae cortex and Safflower seed extract mixture, ^a mean ± SD, * p<0.05 vs control, †p<0.05 vs MCE, ‡p<0.05 vs

SPSS version 8.0

2 -

tailed unpaired t - test

95%

가

III.

(Table 1).

가

(1)



Figure 1. Histologic finding of defect sites of control group at 4 weeks. : New bone formation is minimal. Defect was filled with fibrous connective tissue(*). Arrows indicate original



Figure 2. Histologic finding of defect sites of ZML group at 4 weeks. New bone formation is minimal only from the margin of the defect. Defect was filled with fibrous connective tissue(*). Arrows indicate original border of defect. Masson

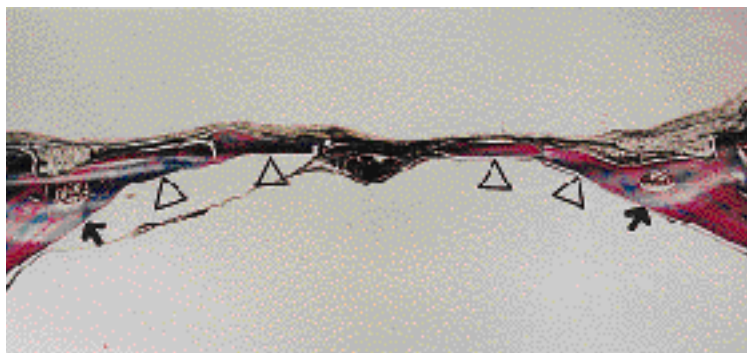


Figure 3. Histologic finding of defect sites of MSM 1 : 5 ratio 0.5g/kg group at 4 weeks. New bone (arrow heads) grows from the margin of the defect toward center. The defect was almost closed with new bone. New bone is thinner than original bone. Arrows indicate original border of defect.



Figure 4. Histologic finding of defect sites of MSM 1 : 10 ratio 0.25g/kg group at 4 weeks. New bone (arrow heads) grow from the margin of the defect. New bone grows more from the left side than the right side of the defect. Arrows indicate original border of defect. Masson trichrome stain.



Figure 5. Histologic finding of defect sites of MSM 1 : 10 ratio 0.5g/kg group at 4 weeks. New bone (NB) grow from the margin of the defect toward center. The defect was almost closed with new bone. New bone is thinner than original bone. Arrows indicate original border of defect.

ALPase ,
 ALPase ,
 , . 1 : 5, 1 : 10 , ,
 ALPase 가 가 1 : 10 가
 ALPase 가 (Table 2). , , .
 , (3)

10 0.1g/kg 0.25g/kg

1 : 1, 1 : 2 , 0.5g/kg 1 : 5 0.25g/kg
, 1 : 10 0.5g/kg

(Table 3). 가

1 : 5, 1 : 10 IV. (Figure 3 - 5).

1 : 10 가 , 1 : 10

(p<0.05). 가

1 : 1 ALPase ,

가 1 : 5, 1 : 10 가 1 : 5, 1 : 10

(4) 8mm 가 4 가

1 : 5 0.5g/kg 가

, 0.25g/kg 1 : 5 가
, 1 : 10 , 0.5g/kg

(Table 4). 가

4 가

(Figure 1), 13-20), Sangunaria²¹⁾

1958 Thiers³⁵⁾ 가
Paget

(Figure 2), 가
1 : 5 0.1g/kg , 1 : 1971 Chaput¹⁴⁾ 1974

Kerebel³⁶⁾ Hamster TGF - ,
 4-6). ,
 가 , 가
 (blood stasis)
 PDGF - BB
 , adenosine diphosphate
 27, 28), 가 , 가
 29) 37).
 ATCC MG63 human osteosarcoma
 cell
 가 ,
 37). magnolol PGE₂
 honokiol 가 가
 (lipid peroxidation)
 - (tocopherol)
 1000 가 ,
 가 가
 30 - 32). V.
 Streptococcus mutans
 가 ,
 , ALPase ,
 가 , IL - 1 가 , 가
 PGE₂ 22 - 가
 26).
 1. 가 ,
 가 ,
 가 가
 PDGF - BB, IGF - 1, 가 . 가

VI.

가 ,
 가 ,
 가 가
 가 1 : 5
 가 .
 2. ALPase ,
 ALPase 가
 ALPase 가
 10 가 1 :
 .
 1 : 5, 1 : 10
 1 : 10 가
 .
 가
 1 : 5, 1 : 10
 가 .
 4. 1
 : 5 , 0.25g/kg
 0.5g/kg , 1 : 10 ,
 0.5g/kg
 ,
 .
 4
 .

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evaluated by histomorphometric measuring of newly formed bone on the 8 mm defect in rat calvaria after oral administration of 2 ratio groups (1 : 5 and 1 : 10) at 3 different doses (0.1, 0.25 and 0.5g/kg per day). tion,

MSM stimulated the growth and survival rate of osteoblasts and PDL cells more than any other agents. The growth and survival rate were increased as the proportion of safflower seed extract was increased. MCE, SSE, ZML stimulated the ALPase activity of osteoblast and PDL cell in comparison to the negative control group. But all groups of MSM regardless of ratio of safflower seed extract stimulated the ALPase activity than any other agent. The ALPase activity was also increased as the proportion of safflower seed extract was increased. Although MCE, SSE, ZML stimulated the proliferation of osteoblasts. 1 : 5 and 1 : 10 ratio MSM showed significant increase in stimulation of proliferation of osteoblasts. No agent significantly increased proliferation of PDL cells. Significant new bone formation were seen where 1 : 5 ratio, 0.5g/kg group and 1 : 10 ratio, 0.25, 0.5g/kg groups were used. These results show that magnoliae cortex extract and safflower seed extract mixture can potentially increase bone regeneration ability.

Key Word : safflower seed(*Carthamus tinctorius* Linne, magnoliae cortex, cellular activity, ALPase activity, bone regenera -