

**ABSTRACT****OSTEOGENESIS ENHANCEMENT BY COMBINATION OF HYDROXY APATITE AND ELLAGIC ACID APPLIED ON BONE DEFECT (STUDY ON RAT *RATTUS NOVERGICUS*)**

Agung Satria Wardhana

**Background:** Bone defect diseases have a high prevalence in Indonesia. Most common therapy for bone defect is bone graft. The graft will initiate osteogenesis on the defect area. Study has prove that phenol compound, as antioxydant and antiinflammation agent, can increase bone formation in bone defect. Current research indicate that Ellagic acid, a phenolic anti-oxidant, have a benefit in healing of bone and wound. Combination between hidroxy apatite and EA is expected to increase osteogenesis process. Osteogenesis can be determined with some mollecular expression, such as osteocalcin, receptor activator nuclear kappa B ligand (RANKL) and osteoprotegerin (OPG). The purpose of this research is to analyze bone defect's osteogenesis after application of combination of hidroxy apatite and 3% ellagic acid through the expression of osteocalcin, RANKL, OPG, osteoblast and osteoclast. **Method:** This is a true experimental research using post-test only with control group design. Our subjects are 30 wistar rats with bone dfect made in left femur, devided in six groups. Each group consist of five rats and divided as: (1) Negative control. Bone defect treated with polyethylene glycol, (2) Positive control treated with hidroxy apatite, and (3) group treated with combination of hidroxy apatite and ellagic acid. The subject sacrificed in day seven anad 14. **Result:** The result shown in treatment group, when compared to control groups, have a significant increase of osteocalcin and OPG expressions ( $P < 0.05$ ), otherwie the expression of RANKL and osteoclast significantly decrease ( $P < 0,05$ ) **Conclusion:** Group with ellagic acid combination show enhancement of osteocalcin, OPG and osteoblas expression, and lower the expression of RANKL and osteoclast.

Keywords: Osteogenesis, hidroxy apatite, ellagic acid, osteocalcin, RANKL, OPG.

## DAFTAR ISI

HALAMAN SAMPUL DALAM .....	ii
LEMBAR PENGESAHAN .....	<b>Error! Bookmark not defined.</b>
PENETAPAN PANITIA PENGUJI TESIS .....	iii
PERNYATAAN ORISINALITAS .....	v
UCAPAN TERIMA KASIH.....	vi
ABSTRAK .....	viii
<i>ABSTRACT</i> .....	ix
DAFTAR ISI.....	x
DAFTAR TABEL.....	xv
DAFTAR GAMBAR .....	xvi
DAFTAR SINGKATAN .....	xvii
BAB 1. PENDAHULUAN .....	1
1.1. Latar Belakang Masalah .....	1
1.2. Rumusan Masalah.....	5
1.3. Tujuan Penelitian .....	5
1.3.1. Tujuan Umum .....	5

1.3.2.	Tujuan Khusus .....	5
1.4.	Manfaat Penelitian .....	5
1.4.1.	Manfaat Teoritis.....	5
1.4.2.	Manfaat Praktis .....	6
BAB 2. TINJAUAN PUSTAKA .....		7
2.1.	Tulang .....	7
2.1.1.	Definisi Tulang .....	7
2.1.2.	Struktur Tulang .....	7
2.2.	Remodeling Tulang.....	10
2.3.	Osteokalsin .....	14
2.4.	<i>Reseptor Activator of Nuclear Factor-Kappa<math>\beta</math> Ligand (RANKL)</i> .....	15
2.5.	<i>Ellagic Acid</i> .....	16
2.6.	Hidroksi Apatit .....	18
2.7.	Rattus Novergicus Strain Wistar.....	20
2.8.	Imunohistokimia .....	21
BAB 3. KERANGKA KONSEP DAN HIPOTESIS PENELITIAN .....		23
3.1.	Kerangka Konseptual Penelitian.....	23

3.2.	Hipotesis Penelitian .....	25
BAB 4. METODOLOGI PENELITIAN.....		26
4.1.	Jenis dan Rancangan Penelitian.....	26
4.2.	Populasi, Besar Sampel dan Teknik Pengambilan Sampel.....	27
4.2.1.	Populasi.....	27
4.2.2.	Teknik Penghitungan Sampel .....	27
4.2.3.	Besar Sampel .....	28
4.3.	Variabel Penelitian.....	28
4.3.1.	Variabel Bebas .....	28
4.3.2.	Variabel Terikat .....	28
4.3.3.	Variabel Terkendali .....	28
4.3.4.	Definisi Operasional .....	29
4.4.	Bahan Penelitian .....	30
4.4.1.	Bahan untuk pembuatan defek tulang pada tikus .....	30
4.4.2.	Bahan untuk perlakuan .....	30
4.4.3.	Bahan untuk pemeriksaan imunohistokimia.....	30
4.5.	Instrumen Penelitian .....	31

4.5.1.	Instrumen untuk pembuatan jejas pada tikus.....	31
4.5.2.	Instrumen untuk perlakuan .....	32
4.5.3.	Instrumen untuk pemeriksaan laboratorium .....	32
4.6.	Lokasi dan Waktu Penelitian .....	32
4.6.1.	Lokasi Penelitian.....	32
4.6.2.	Waktu Penelitian.....	32
4.7.	Prosedur Penelitian dan Pengumpulan Data.....	32
4.7.1.	Perijinan Ethical clearance.....	32
4.7.2.	Pengelompokan Hewan Coba.....	33
4.7.3.	Pembuatan HA dan kombinasi HA + EA .....	33
4.7.4.	Pembuatan Luka pada hewan coba.....	33
4.7.5.	Pengorbanan dan pengambilan jaringan .....	34
4.7.6.	Pembuatan Sediaan .....	34
4.7.7.	Teknik Pengecatan Imunohistokimia dan Penghitungan.....	37
4.7.8.	Penanganan Bangkai Hewan Coba.....	38
4.8.	Bagan Alur Operasional Penelitian.....	39
4.9.	Analisis Data.....	40

BAB 5. HASIL PENELITIAN .....	41
5.1. Hasil Penelitian .....	41
5.1.1. Uji Imunohistokimia .....	41
5.1.2. Perhitungan Jumlah Osteoblas dan Osteoklas .....	45
5.2. Analisis hasil penelitian .....	47
BAB 6. PEMBAHASAN .....	54
BAB 7. KESIMPULAN DAN SARAN .....	61
7.1. Kesimpulan .....	61
7.2. Saran .....	61
DAFTAR PUSTAKA .....	62
LAMPIRAN-LAMPIRAN.....	67

**DAFTAR TABEL**

	Halaman
Tabel 5.1 Ekspresi Osteokalsin.....	42
Tabel 5.2 Ekspresi RANKL.....	43
Tabel 5.3 Ekspresi OPG .....	45
Tabel 5.4 Ekspresi Osteoblas.....	46
Tabel 5.5 Ekspresi Osteoklas.....	47
Tabel 5.6 Rerata ekspresi Osteokalsin, RANKL, OPG.....	48
Tabel 5.7 Rerata jumlah osteoblas dan osteoklas .....	48
Tabel 5.8 Hasil uji normalitas data .....	49
Tabel 5.9 Hasil uji homogenitas data .....	49
Tabel 5.10 Hasil Uji post-hoc LSD Osteokalsin .....	50
Tabel 5.11 Hasil Uji post-hoc LSD RANKL .....	51
Tabel 5.12 Hasil Uji post-hoc LSD OPG .....	51
Tabel 5.13 Hasil Uji post-hoc LSD Osteoblas .....	52
Tabel 5.14 Hasil Uji post-hoc LSD Osteolas .....	53

## DAFTAR GAMBAR

	Halaman
Gambar 2.1 Proses penyembuhan tulang.....	11
Gambar 2.2 Struktur kimia ellagic acid.....	16
Gambar 2.3 Struktur kimia hidroksi apatit.....	18
Gambar 3.1 Kerangka konsep penelitian .....	23
Bagan 4.1 Bagan Rencana Penelitian .....	25
Gambar 4.1 Alur Penelitian .....	39
Gambar 5.1 Ekspresi Osteokalsin .....	42
Gambar 5.2 Ekspresi RANKL .....	43
Gambar 5.3 Ekspresi OPG .....	44
Gambar 5.4 Ekspresi Osteoblas dan Osteoklas .....	46



## DAFTAR SINGKATAN

HA	: Hidroksi Apatit
EA	: Ellagic acid
RANKL	: <i>Receptor Activator of Nuclear Factor Kappa <math>\beta</math> Ligand</i>
OPG	: Osteoprotegerin
OPN	: Osteopontin
CFU	: Colony Formation Unit
CFU-F	: Colony Formation Unit Fibrosit
CFU-GM	: Colony Formation Unit Granulosit Makrofag
TGF- $\beta$	: Transforming Growth Factor
TNF	: Tumor Necrose Factor
OPGL	: Osteoprotegerin Ligand
ODF	: Osteoklas <i>Differentiation Factor</i>
TRANCE	: TNF Related Activation Induced Cytokine
RANK	: <i>Receptor Activator of Nuclear Factor Kappa <math>\beta</math></i>
M-CSF	: <i>Macrophage Colony Stimulating Factor</i>
PEG	: Poly Ethylene Glycol
RA	: Random Allocation
HE	: Haematoxillyn Eosin
PBS	: Posphate Buffer saline
DMSO	: <i>Dimethyl sulfoxide</i>
EDTA	: Ethylene Diamine Tetra Acetil Acid
BHK-21	: Baby Hamster Kidney