International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.06 pp 686-693, 2016

Osteogenesis at Tension Site by *Stichopus hermanii* Application as Relapse Orthodontic Prevention

Noengki Prameswari[1]*, Soetjipto[2], Retno Pudji Rahayu[3]

[1]Faculty of Dentistry, Hang Tuah University, Surabaya, Indonesia
[2]Faculty of Medicine, Airlangga University, Surabaya, Indonesia
[3]Faculty of Dentistry, Airlangga University, Surabaya, Indonesia

Abstract: Objective : The aim of this study is to investigate osteogenesis at tension site by *Stichopus hermanii* application as relapse orthodontic prevention.
Materials and Methods: The experiment was held by Post Test Only Group design. Thirty two male Cavia Cobaya were divided into four groups. K(-) group as negative control group (without treatment), K(+) group as positive control group which were applied with relaps orthodontic forces, and the other groups P1, P2, were applied with relaps orthodontic forces and Stichopus hermanii 2,5 % and 3 %. After treatment the cavia cobaya were sacrificed. Collagen type1 and BMP-2 expression at tension site as osteogenesis marker were examined with immunohistochemistry.
Results: This study showed collagen type 1 and BMP-2 expression especially in P2 increased to show osteogenesis occured compare with K(-), K(+), and P1.
Conclusion : Osteogenesis occurred at tension site by Stichopus hermanii as relapse orthodontic prevention. 3 % Stichopus hermanii is the effective dose for osteogenesis.
Keywords: Stichopus hermanii, Ostegenesis, relapse orthodontic prevention.

Introduction:.

Orthodontic treatment are potentially unstable although a number of factors can be cited as influencing long term-result. It called relapse orthodontics. Relapse Orthodontic is the return, following correction, any change from the final tooth position at the end of treatment relapse, could be a return to the original teeth position, caused by periodontal, occlusal, soft tissue factor and growth. Orthodontic relapse is a complex problem. [1,2] Studies have shown that stability and relapse following orthodontic treatment are unpredictable, with a relapse tendency of 33–90 per cent after at least 10 years post-treatment.[3]

One major reasons is the gingival and periodontal tissues are affected by orthodontic tooth movement and require time for reorganization when appliance are removed.[1,2] Stretching of supraalveolar gingival fibres, the transseptal fibres, in particular, has been suggested as the cause of relapse. [4] Orthodontic tooth movement is achieved following remodelling of the alveolar bone and a reaction of the periodontal ligament (PDL) to mechanical stimuli. Tooth movement occurs in the direction of force when there is a multifaceted bone remodelling response, with bone resorption on the compression side and bone apposition on the tension side of the periodontal ligament and alveolar bone.[5] The periodontal ligament reorganization and bone remodeling is important for stability because of the periodontal contribution to the equilibrium that normally controls tooth position. [1]

Relapse occurred in all appliance following the end of active orthodontic treatment. There was a rapid relapse initially following appliance removal but after 3 days, both relapse rate and the percentage of relapse began to gradually decrease. After appliance removal, the teeth began to relapse in the direction of their original position; this reverse tooth movement being accompanied by an alteration in the number and distribution of osteoclasts. The number of osteoclasts declined significantly in both mesial and distal roots of the first molar within 3 days, most probably as a result of apoptosis and/or decreased blood vessel density.[3]

Instability or a tendency toward relapse should be anticipate. Patients should be advised of potential for relapse prior treatment and the need to stay in long-term retention.[2] Beside retention, fiberotomy is also known for diminished relapse.[6]

Many but there is no natural has been used for relapse orthodontics.Stichopus hermanii is one of the best fishery commodities in Indonesia. It is natural and contain various active ingredient such as hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid[7] that might reduce relapse orthodontic. Previous research showed that *Stichopus hermanii* modulated the inflammatory responses, stimulated the activation and proliferation of fibroblasts, and enhanced rapid production of collagen fiber network with shorter healing time. The level of proinflammatory cytokines; IL-1α, IL-1β, and IL-6, were significantly reduced in Stichopus hermanii treated wounds and stimulation tissue regeneration.[8] The other study show that studies have shown that the extract of Stichopus species also affects viability or proliferation of human fibroblasts and osteoclast cells in a negative manner.[9] In this study, we would to investigate ostegenesis at tension site by giving *Stichopus hermanii* in relapse orthodontic.

Material and Method:

This study was performed on 32 male *Cavia cobaya* 2,5 months old with 200-300 g weight. Ethical Approval for this research was obtained from Ethical committee of Dentistry Faculty Airlangga University in April 2015. The 32 male Cavia cobaya was divided into 4 groups. K(-) group as negative control group (without any treatment), K(+) group as positive control group which were applied with separator rubber for resulting relaps orthodontic forces, and the treatment groups P1, P2, were applied with relaps orthodontic forces and *Stichopus hermanii* 2,5 % and 3 %.

Preparation of Relapse orthodontic

Relaps orthodontic forces was produced with giving applied separator rubber by separating plier in mesial left insisivus maxilla cavia cobaya 14 days and after day 15 separator was removed for 7 days for becoming relapse orthodontic. Separator forces was 0,0474 kN, measured by autograph.

Preparation of Powder *Stichopus hermanii*

Stichopus hermanii were used in this study from coastal regions around Sumenep, East Java Indonesia. *Stichopus hermanii* was cleaned by making a longitudinal incision 3-5 cm on the ventral side of stichopus hermanii without damaging the internal organs using scalpel. *Stichopus hermanii* was dried in oven 28°C for 7 days. After this, *Stichopus hermanii* was blender until get the powder.

Preparation and Applied *Stichopus Hermanii* gel

Stichopus hermanii gel 2,5% was made from 0,25 gr *Stichopus hermanii* powder was diluted with NaCMC 2% in DMSO 5 % until 10 ml. Stichopus hermanii gel 3% was made from 0,3 gr *Stichopus hermanii* powder was diluted with NaCMC 2% in DMSO 5 % until 10 ml. *Stichopus hermanii* gel was applied in gingival sulcus with insulin syringe 0,025 ml twice per day.

The research was conducted in Biochemistry Laboratory Medical Faculty of Airlangga University. After 21 days, the *Cavia cobaya* were sacrificed. The jaw was sectioned. Collagen type 1 and BMP-2 expression as osteogenesis marker were examined with immunohistochemistry method in tension side. The research was analyzed by ANOVA test (analysis of varians) and LSD Test.

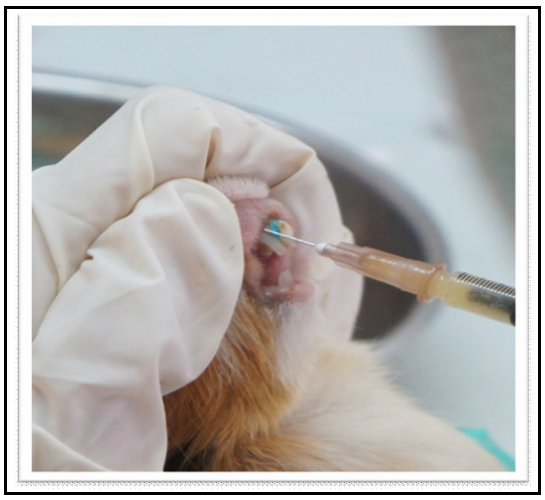

Fig 1 : Stichopus hermanii gel was applied in gingival sulcus with insulin syringe

Results:

The aim of this study is to investigate osteogenesis at tension site relapse orthodontic by Stichopus hermanii. The result in this experiment showed collagen type I expression as shown as fig 2 and BMP-2 expression as shown as fig 3.

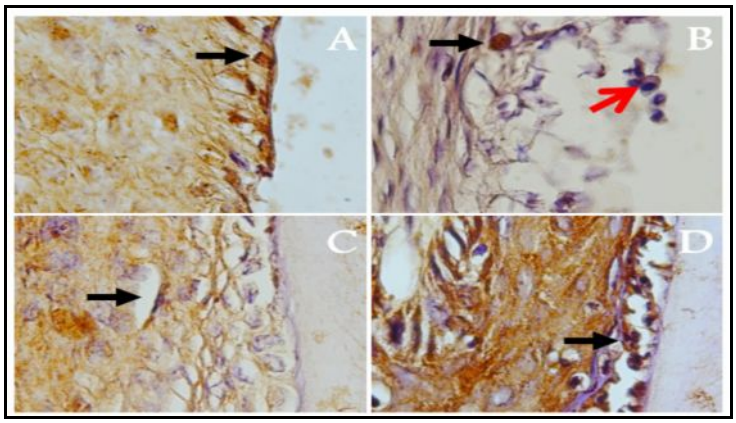

Fig 2 : Photomicrograph of collagen type 1 expression in K(-), K(+), P1, and P2

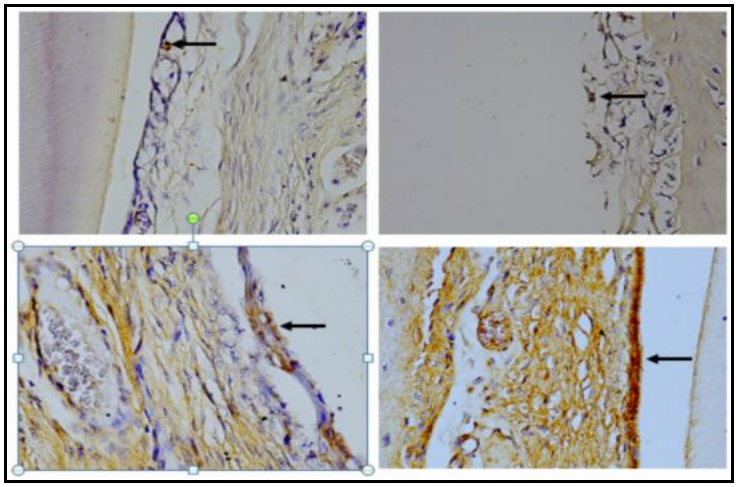

Fig 3 : Photomicrograph of BMP-2 expression in K(-), K(+), P1, and P2

Table 1 : The Expression of collagen type 1 in relaps orthodontics *Cavia cobaya* applied with *Stichopus hermanii*

Group	Mean± Standart Deviation
K(-)	16,70±2,54
K(+)	8±2,07
P1	21±1,00
P2	26,25±2,38

Table 2 : LSD Test expression of collagen type 1 in relaps orthodontics *Cavia cobaya* applied with Stichopus hermanii

Group	K(-)	K(+)	P1	P2
K(-)		0,000	0,000	0,000*
K(+)	0,000		0,000*	0,000*
P1	0,000	0,000*		0,000*
P2	0,000*	0,000*	0,000*	

Table 1 showed means and SD in K(-), K(+), P1,and P2 are 16,70±2,54; 8±2,07; 21,1±1,0 and 26,25±2,38.Then the data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. ANOVA test (p=0.05) for the expression of collagen type 1 activity in relaps orthodontics *Cavia Cobaya* applied with *Stichopus hermanii* showed significantly differences. With the LSD test as seen as table 2, showed that P1 and P2 showed increased collagen type 1 expression whether P2 has the best expression. So, the expression of collagen type 1 was significantly increased in P2 compare to K(+), K(-) and P1.

Table 3 : The Expression BMP-2 as osteoblast activity in relaps orthodontics Cavia Cobaya applied with Stichopus hermanii

Group	Mean± Standart Deviation
K(-)	7,13±2,17
K(+)	3,75±1,98
P1	13,38±2,5
P2	21±1,3

Table 4 : LSD Test expression BMP-2 as osteoblast activity in relaps orthodontics Cavia Cobaya applied with Stichopus hermanii

Group	K(-)	K(+)	P1	P2
K(-)		0,019	0,003	0,000*
K(+)	0,019		0,000*	0,000*
P1	0,003	0,000*		0,000*
P2	0,000*	0,000*	0,000*	

*Significantly different

Table 3 showed means and SD in K(-), K(+), P1,and P2 are 7,13±2,17; 3,75±1,98; 13,38±2,5 and 21±1,3.Then the data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. ANOVA test (p=0.05) for the expression of BMP-2 as osteoblast activity in relaps orthodontics Cavia Cobaya applied with Stichopus hermanii showed significantly differences. With the LSD test as seen as table 4, showed that P1 and P2 showed increased BMP-2 expression whether P2 has the best expression. So, the expression of BMP-2 was significantly increased in P2 compare to K(+), K(-) and P1.

Discussion

The aim of this study is to investigate osteogenesis at tension site by applying Stichopus hermanii in relapse orthodontic. In relapse orthodontic, there are decreasing collagen type 1 expression as marker of bone matrix compare to K (-), P1 and P2. By applying Stichopus hermanii 2,5 % and 3 % in gingival sulcus tension site, collagen type 1 was increased. P1 and P2 showed increased collagen expression whether P2 (stichopus hermanii 3 %) has the best expression in osteogenesis as seen as fig 2.

The means and standar deviations of BMP-2 as marker of osteoblast in relapse orthodontic showed that there are decreasing BMP-2 expression compare to K (-), P1 and P2. By giving Stichopus hermanii 2,5 % and 3 % in gingival sulcus tension site, BMP-2 was increased. P1 and P2 showed increased BMP-2 expression whether P2 (Stichopus hermanii 3 %) has the best expression as seen as fig 3.

Relapse in cavia cobaya models occurs rapidly. The left first insisivus compressed towards the distal side during 14 days orthodontic tooth movement and relapsed toward the mesial side. There was a rapid relapse initially following 2 days appliance removal. Osteogenesis is needed for preventing orthodontic relapsed movement to the original position before orthodontic treatment. BMP-2 as marker osteoblast activity showed increasing by applying Stichopus hermanii. Osteoblast activity was increased proved that there are bone apposition. In Bone remodeling theory, increasing osteoblast activity begins when osteoclast activity was decreased.

Stichopus hermanii have high commercial value with increasing global production and trade. Therapeutic properties and medicinal benefits of Stichopus hermanii can be linked to the presence of a wide array of bioactives. Stichopus hermanii contain nutrients such as Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin), and minerals, especially calcium, magnesium, iron and zinc. A number of unique biological and pharmacological activities including anti-angiogenic, anticancer, anticoagulant, anti-hypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor and wound healing have been ascribed to various species of Stichopus. Therapeutic properties and medicinal benefits of Stichopus hermanii can be linked to the presence of a wide array of bioactives especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, cerberosides, lectins, peptides, glycoprotein, glycosphingolipids and essential fatty acids. [10] Stichopus hermanii contain various active ingredient that usefull in bone remodelling such as hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid.[7] Stichopus hermanii propose a potent anti-osteoclastogenic effect because hyaluronan. Chondroitin sulphate also has potent antiosteoclastogenic effect. Condroitin Sulphate on the surface of osteoblasts or bone matrix binds to cell adhesion molecule such as integrin on the pre-osteoclastic cells and inhibits the differentiation into osteoclasts. [11,12] EPA DHA also a potent inhibitor osteoclastogenesis. EPA DHA was found to inhibit osteoclast differentiation, activation and function. EPA DHA inhibit osteoclast-specific genes like tartrate resistant acid phosphatase. [13] Flavonoid in previous study was also decreases osteoclastic differentiation, by inhibiting RANKL-induced nuclear factor kB (NF kappa B) and activator protein 1 (AP-1) activation. NF kappa B and AP-1 are transcription factors highly involved in osteoclastic differentiation and their inhibition could play an important role in the decrease of osteoclastogenesis and osteogenesis begins.[14] Flavonoid inhibited osteoclastogenic factors and osteoclast formation in bone marrow-derived macrophages and osteoblast co-cultured cells, and increased osteoprotegerin (OPG) levels in osteoblasts.[15]

Osteoclast reduction induced bone formation with osteopontin is required to convey the effect of mechanical stress to osteoblast. Osteopontin directly modulates bone formation in the response to mechanical stress independent of its effect of osteoclast.[16] Bone formation could be resistant to resorption and countered the relaps orthodontic. Osteogenesis consist of osteoblast proliferation, differentiation, and collagen have role in bone matrix filling and mineralization.[17] Collagen type I is the resulting expression of the highest collagen (90%) of osteoblasts.[18]

Stichopus hermanii also contain various active ingredient such as collagen, hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid. [7] Previous studies showed that the water extract of Stichopus contains high amino acid concentrations (37%), 34% as well as calcium, magnesium, iron and zinc. Collagen itself have function to modulate the formation of bone matrix in osteogenesis.[9] The effect of glycosaminoglycan (GAG) such as chondroitin sulphate, oral administration had been shown to increase the total calcium pool and

intestinal absorption of calcium, which may lead to an increased capacity for injured bone to regenerate during osteogenesis.[9] Chondroitin Sulphate (CS) is an unbranched long chained-heteropolysacharide called glycosaminoglycans (GAGs). GAGs is a heteropolysacharide possessing negative edge binding protein called mucopolysacharide. CS is major component of the extracellular matrix which plays a role in maintaining the structural integrity of the tissue.[19] Condroitin Sulphate on the surface of bone matrix binds to cell adhesion molecule such as integrin.[12]

The bone morphogenetic proteins (BMPs) included BMP-2, is the second family of growth factors, unique: these are the growth factors involved in the process of osteoblast differentiation that drive the process of bone formation and mineralization. Since the late 1980s, BMPs have been known to stimulate osteogenesis. BMPs represent molecular targets used to identify and develop new agents to simulate the bone-forming process. Much is understood about the signal transduction pathway for the BMPs. BMP-2 stimulates the differentiation of mesenchymal cells into osteoblasts and chondrocytes. BMP-2 binds to its receptor, a Ser/Thr kinase, which phosphorylates and activates the intracellular signaling molecules Smad 1 and Smad 5. This in turn leads to the expression of the transcription factor Cbfa1 (Runx2), which results in the expression of several proteins critical for bone formation. Wnt/LRP5 pathway is also linked to the BMP pathway by a cascade of anabolic transcriptional events. The signal starts at the Hedgehog signaling pathway, moving through the BMPs and Wnt/LRP5, and ultimately leads to expression of the critical genes involved in osteoblast differentiation. This pathway provides multiple potential molecular targets that may be manipulated in the process of bone formation in osteogenesis. [20] The process that been needed to prevent orthodontic relapsing

Stichopus hermanii contain various active ingredient such as collagen, hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid.[7] In a previous in-vitro study showed that there was a positive promoting effect of stichopus hermanii water extract on osteoblast functional activity when 1.6mg/ml, 3.1mg/ml, 6.3mg/ml, 12.5mg/ml, and 25mg/ml of stichopus hermanii concentrations were used. Microscopic examination showed adequate cell confluency in the wells with stichopus hermanii concentration from 1.6 mg/ml up to 25mg/ml. Previous studies showed that the water extract of Stichopus contains high amino acid concentrations (37%) as well as calcium, magnesium, iron and zinc that may play an important role in osteoblast molecular activities. [9] Calcium in sufficient amount are very effective, especially prior to achieve bone formation and maximum density of bone.[21,22] Vitamis influencing early regeneration of all connective tissues involved in the healing and quicker mineralization.[23]

The effect of glycosaminoglycan (GAG) such as chondroitin sulphate, oral administration had been shown to increase the total calcium pool and intestinal absorption of calcium, which may lead to an increased capacity for injured bone to regenerate during osteogenesis.[9] Condroitin Sulphate on the surface of osteoblasts or bone matrix binds to cell adhesion molecule such as integrin on the pre-osteoclastic cells and inhibits the differentiation into osteoclasts so bone formation can occurred.[12]

Stichopus hermanii also has flavonoid as its content. Flavonoid has been reported to have some biological activities such as anti-inflammatory, antibacterial, antiviral, anti-allergic, antitumor, neurodegenerative and vasodilatory effect.[24] Flavonoid as antioxidant can protect cell from damage. One that caused cell damage is mechanical stress from relaps orthodontic. [25] Its have function Flavonoid stimulates human osteoblast differentiation. In vivo, flavonoid increases bone mass in immobilized rats and also the biomechanical properties of rat bone.[20] Flavonoid treatment resulted in a significant elevation of alkaline phosphatase (ALP) activity, collagen contents and osteoblast differentiation genes [ALP, collagen, osteopontin (OPN), osteoprotegerin (OPG) and osteocalcin (OC)] and bone morphogenetic protein (BMP) genes (BMP2, BMP4 and BMP7).[26, 27] Flavonoid activated BMP signaling by inducing Smad1, 5 phosphorylation, as well as Id1 and Id2 protein expression in a dose-dependent manner. [26] Osteogenesis could be resistant to resorption and countered the relaps orthodontic.[17]

Conclusion:

- Stichopus hermanii 3 % can increase osteogenesis at tension site relapse orthodontic that could be resistant to resorption and countered the relapse orthodontic.

- Stichopus hermanii 3% application have active ingredients such as collagen, hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid that can increase expression of BMP-2, Collagen type 1 that have role in osteogenesis as relapse orthodontic prevention .

Acknowledgement

This research was supported by a grant from Unggulan Research Program, funded by Ministry of Education and Culture Indonesia 2015-2016.

References

1. Proffit WR, Fields HW. Sarver DM. 2007. Contemporary Orthodontic. 4th edition. Mosby Elsevier. Missouri.
2. English J. 2009. Orthodontic Review. 1st edition. Mosby Elsevier.Missouri.
3. Franzen, TJ, Brudvik P, Radunovic VV. 2011. Periodontal Tissue Reaction during Orthodontic Relapse in Rat Molars. European Journal of Orthodontics edisi Oktober 2011 Available at : European J http://ejo.oxfordjournals.org/content/early/2011/10/24/ejo.cjr127.full#ref-11.
4. Yoshida Y, Sasaki T, Yokoya K, Hiraide T, Shibasaki Y. 1999. Cellular Roles in Relapse Processes of Experimentally-moved Rat Molars. Electron Microsc 1998(48): 147-157.
5. Krishnan V, Davidovitch Z. 2006. Cellular, Molecular, and Tissue-level reactions to Orthodontic Force. American Journal of Orthodontics and Dentofacial Orthopedics .Vol 129. No 4 : 469.
6. Young L, Binderman I, Yaffe A, Beni L, Vardimon AD. 2013. Fiberotomy Enhances Orthodontic Tooth Movement and diminishes Relapse in a Rat Model. Orthod Craniofac Res vol 16(3):161-8.
7. Sendih, Skolastika, Gunawan, 2006. Keajaiban Teripang Penyembuh Mujarab Dari Laut. Jakarta: Agro Media Pustaka.
8. Zohdi RM, Zakaria ZA, Yusof N, Mustapha NM, Abdullah MN. 2011. Sea Cucumber (Stichopus hermanii) based Hydrogel to Treat Burn Wounds in Rats. Journal Biomed Mater Res vol 98(1): 30-7.
9. Shahrulazua A, Samsudin AR, Iskandar MA, Amran. 2013. The In In-Vitro Effects of Sea Cucumber (Stichopus sp1) Extract on Human Osteoblast Cell Line. Malaysian Orthopaedic Journal 2013 Vol 7 No 1.
10. Revianti S, Soetjipto, Rahayu RP, Parisihni K. 2016. Protective role of Sticophus hermanii ethanol extract supplementation to oxidative stress and oral hyperkeratosis in smoking exposed rats. International Journal of ChemTech Research Vol.9 (05): 408-417.
11. Chang EJ, Kim HJ, Ha J, Kim HJ, Ryu J, Park KH, Kim UH, Lee ZH, Kim HM, Fisher DE, Kim HH. 2007. Hyaluronan inhibits Osteoclast Differentiation via Toll-like receptor 4. Journal Cell Sci Vol 120 (Pt 1) : 166-76.
12. Miyazaki,2010. Effect of Chondroitin sulphate-E on the Osteoclastic Differentiation of RAW264 cells. Dental Materials Journal vol 29(4): 403-10.
13. Rahman MM, Bhattacharya A, Fernandes G. Docosahexaenoic Acid is more potent inhibitor of Osteoclast Differentiation in RAW 264.7 cells than Eicosapentaenoic Acid. J Cell Physiol Vol 214(1):201-9.
14. Wattel A, Kamel S, Prouillet C, Petit JP, Lorget F, Offord E, Brazier M. 2004. Flavonoid quercetin decreases Osteoclastic Differentiation induced by RANKL via a mechanism involving NF kappa B and AP-1. Journal of Cell Biochem vol 92(2): 285-95.
15. Huh JE, Jung IT, Choi J, Baek YH, Lee JD, Park DS, Choi DY. 2013. The natural flavonoid galangin inhibits Osteoclastic Bone Destruction and Osteoclastogenesis by Supressing NF-kB in Collagen-induced Arthritis and Bone Marrow-derived Macrophages. Eur J Pharmacol Vol 698(1-3):57-66.
16. Ishijima M, Rittling SR, Yamashita T, Tsuji K, Kurosawa H, Nifuji A, Denhardt DT, Noda M. 2001. Enhancement of Osteoclastic Bone Resorption and Suppression of Osteoblastic Bone Formation in Response to Reduced Mechanical Stress Do Not Occur in the Absence of Osteopontin. J. Exp. Med. Vol 193 (3):399-404.
17. Shetty S, Mogra S, Shetty S. 2006. The Effect of the Short Term Administration of a Biphosphonate upon Dental Relapse. J Ind Orthod Soc vol 39:198-203.
18. Isparnadi E, Hidayat M, Aulanni'am, Permatasari N. 2015. Study of osteocalcin and collagen type I in Regeneration of Atrophic Non-Union Fracture based on Bivalve Anodonta-PRP Formula and

Mesenchymal Stem Cells as Composite Scaffold in Regeneration of Atrophic Non-Union Fracture. International Journal of ChemTech Research Vol.8 (3): 1041-1046.

19. Sulistyowati W, Titiek IA, Arsiniati A, Erina Y. 2015. Glucosamine and Chondroitin Sulphate Content of Shark Cartilage (Prionace glauca) and its Potential as Anti-Aging Supplements. International Journal of ChemTech Research. Vol 8 (10):163-168.
20. Mundy GR. 2006. Nutritional Modulators of Bone Remodeling during Aging. Am J Clin Nutrition Vol 83 No 2 : 4275-4305.
21. Talib A, Suprayitno E, Aulani'am, Hardoko. 2014. Therapeutic Dose of Madidihang Fish Bone Flour and CaCO3 towards Calcium and Phosphorus Content in Blood Serum and Bones of Ovariectomy Rat.. International Journal of ChemTech Research. Vol.6(14): 5529-5534.
22. El-Haliem SA, El-Sherif, El-Ghafour SA. 2016. Investigation of the Quality Properties and Nutritional Values of Four Fish Species from Lake Qaroun, Egypt. International Journal of ChemTech Research Vol.9 (04): 16-26.
23. Mishra G, Srivastava S, Nagori BP. 2010. Pharmacological and Therapeutic Activity of Cissus quadrangularis: An Overview. International Journal of PharmTech Research Vol.2, (2): 1298-1310.
24. Malik A, Ahmad AR. 2014. Determination of Phenolic and Flavonoid Contents of Ethanolic Extract of Kanunang Leaves (Cordia myxa L.). International Journal of PharmTech Research. Vol 7 No 2: 243-246.
25. Alrawaiq NS, Abdullah A. 2014. Review of Flavonoid Quercetin: Metabolism, Bioactivity and Antioxidant Properties. International Journal of PharmTech Research Vol.6(3): 933-941.
26. Kim HS, Suh KS, Ko A, Sul D, Choi D, Lee SK, Jung WW. The Flavonoid glabridin attenuates 2-deoxy-D-ribose-induced Oxidative Damage and Cellular Dysfunction in MC3T3-E1 Osteoblastic Cell. International Journal of Molecular Medicine. Vol 31 No 1: 1107-3756
27. Vrijens K, Lin W, Cui J, Farmer D, Low J, et al. 2013. Identification of Small Molecule Activators of BMP Signaling. PLoS ONE 8(3): e59045.

*****