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The Effect of Palm Tocotrienol on Surface Osteoblast and Osteoclast in Excess Glucocorticoid Osteoporotic Rat Model

(Kesan Tokotrienol Sawit kepada Permukaan Osteoblas dan Osteoklas dalam Model Tikus Berlebihan Osteoporosis Glukokortikoid)

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ABSTRACT

A balanced interaction between osteoblast and osteoclast plays a major role in maintaining bone strength and structural integrity. Tocotrienol, a palm derivative with potent antioxidant properties showed an anti-osteoporotic effect but little is known about its mechanism of action. Hence, this research was conducted to determine the effects of palm tocotrienol on the surface osteoblast and osteoclast of the the glucocorticoid-induced osteoporotic bones. The study involved 40 male Sprague-Dawley rats weighing 250-300 g which were randomly divided into an equal number of Baseline, Sham, Adrx+Dexa (adrenalectomized with dexamethasone), Adrx+Dexa+ATF (adrenalectomized with dexamethasone supplemented with α-tocopherol) and Adrx+Dexa+PTT (adrenalectomized with dexamethasone supplemented with palm tocotrienol). Bloods were taken prior to sacrifice for serum biomarkers and either tibia or femur was harvested for bone biomarkers, gene expressions analysis and histological studies. A double-blinded experiment was performed to calculate the number of total cells (osteoblasts and osteoclast) with intact nuclei within five fields of view. While serum osteocalcin and gene expression did not show any changes, CTX level was increased in the Adrx+Dexa group but reduced after the supplementation of palm tocotrienol. Supplementation of palm tocotrienol also significantly decrease the osteoclast population ($p<0.05$) compared to Adrx+Dexa group. In contrast, osteoblast population showed no significant difference across the groups. The result showed that palm tocotrienol acts by modulating the osteoclasts but not osteoblast, which revert the dynamics of bone cells population in the osteoporotic bone to its pre-osteoporotic levels. Supplements of tocotrienol in daily diet may be beneficial in preventing osteoporosis.

Keywords: Antioxidant; osteoblast; osteoclast; osteoporosis; vitamin E

ABSTRAK

Interaksi seimbang antara osteoblas dan osteoklas memainkan peranan utama dalam mengekalkan kekuatan dan integriti struktur tulang. Tokotrienol, terbitan sawit dengan sifat antioksidan yang kuat telah menunjukkan kesan anti-osteoporosis tetapi amat sedikit diketahui tentang mekanisme tindakannya. Oleh yang demikian, kajian ini dijalankan untuk menentukan kesan tokotrienol sawit ke atas sel permukaan osteoblas dan osteoklas pada tulang osteoporosis aruhan glukokortikoid. Kajian ini melibatkan 40 ekor tikus Sprague-Dawley jantan dengan berat antara 250-300 g yang dibahagikan secara rawak kepada kumpulan Kawalan, Sham, Adrx+Dexa (adrenalektomi dengan deksamethason), Adrx+Dexa+ATF (adrenalektomi dengan deksamethason ditambah α-tokoferol) dan Adrx+Dexa+PTT (adrenalektomi dengan deksamethason ditambah tocotrienol sawit) secara oral selama dua bulan. Darah diambil daripada tikus sebelum dikorbankan dan tulang tibia atau femur kemudiannya diambil untuk kajian petanda biokimia dan histologi. Kajian 'double blinded' dilakukan dengan menghitung jumlah keseluruhan sel (osteoblas dan osteoklas) dengan nukleus dalam lima medan pandangan. Sementara serum osteokalsin dan ekspresi gen tidak menunjukkan sebarang perubahan, aras CTX meningkat pada kumpulan Adrx+Dexa tetapi menurun dengan suplementasi tocotrienol sawit. Suplementasi tocotrienol sawit juga menyebabkan penurunan ketara bilangan osteoklas ($p<0.05$) berbanding kumpulan Adrx+Dexa. Sebaliknya, populasi osteoblas tidak menunjukkan perbezaan yang signifikan pada kesemua kumpulan. Keputusan kajian menunjukkan bahawa tokotrienol sawit bertindak dengan memodulasi osteoklas tetapi bukan osteoblas yang mengembalikan dinamik osteoblas dan populasi osteoblas dalam tulang osteoporosis ke tahap pra-osteoporosis. Tambahan tokotrienol dalam diet harian mampu memberi manfaat dalam mencegah osteoporosis.

Kata kunci: Antioksidan; osteoblas; osteoklas; osteoporosis; vitamin E

Introduction

Osteoblasts are mesenchymal derived from mesodermal and neural crest progenitors that involved in bone formation (Khosla et al. 2008). Most of the bone matrix proteins such as type I collagen, proteoglycans and osteocalcin are secreted and deposited by mature osteoblasts. Osteoblasts are also responsible in regulating the formation of hydroxyapatite crystals in osteoid. Conversely, the differentiation of monocyte-macrophage precursor cells at the bone matrix created a tissue-specific macrophage polykaryon called osteoclast. Osteoclast undergoes internal structural changes that prepare it to resorb bone by rearrangements of the actin cytoskeleton and formation of a tight junction between the bone surface and basal membrane to form a sealed compartment (Boyle et al. 2003). These changes occur in response to activation of receptor activator of nuclear factor kappa-B ligand (RANKL) (Clarke 2008).

The interaction between osteoblasts and osteoclasts is known as coupling and it is essential for maintaining a balance between the rates of bone formation and resorption (Lemaire et al. 2004). Imbalanced activity between osteoclasts and osteoblasts can arise from various factors such as hormonal changes, enhanced production of inflammatory cytokines or growth factors. These can result in either decreased or increased bone mass. While the increased in bone mass can be seen in osteopetrosis, a heterogeneous group of rare heritable conditions, bone loss is far more prevalent and observed in many diseases such as in chronic infections, periodontitis, rheumatoid arthritis, leukemia, postmenopausal osteoporosis and lytic bone metastases (Leibbrandt & Penninger 2008).

Osteoporosis is a skeletal condition characterized by decreased density of normally mineralized bone that impairs the mechanical strength of the bone and making it vulnerable to fracture (Glaser & Kaplan 1997). There are two types of osteoporosis which are primary and secondary osteoporosis. Primary osteoporosis is mainly a skeletal disorder of postmenopausal women (postmenopausal osteoporosis) or of older men and women (senile osteoporosis). Meanwhile, secondary osteoporosis refers to bone loss resulting from specific clinical disorders such as thyrotoxicosis or hyperadrenocorticism (Marcus 1996). Bone loss and eventually fractures are the hallmarks of osteoporosis, regardless of the underlying cause or causes. The excessive activity of osteoclast and inadequate activity of osteoblast are the responsible pathology of the imbalance between the formation and resorption of bone in postmenopausal and age related osteopenia (Manolagas 2000).

Recent studies have shown that free radicals and lipid peroxidation play an important role in bone metabolism especially in osteoblast and osteoclast activity. These free radicals are found to be cytotoxic to osteoblast. As a potent antioxidant, vitamin E was able to maintain bone matrix trophism and stimulate trabecular bone formation. It was supported by other studies which showed that bone impairment caused by an oxidizing agent, ferric nitrilotriacetate, can be prevented by vitamin E supplementation (Maniam et al. 2008). Vitamin E exists in the form of tocopherols and tocotrienols. Both have several type of isomers known as alpha, beta, gamma and delta (Traber 2007). Structurally, tocopherols consist of a chromanol ring and a 15-carbon tail derived from homogentisate (HGA) and phytyl diphosphate. Tocotrienols differs from tocopherols by the presence of three trans double bonds in the hydrocarbon tail. The primary form of tocotrienols in vitamin E is found in the seed endosperm of most monocots, such as wheat, rice and barley (Sen et al. 2006). Tocotrienol are found more abundant in palm, rice and annatto fruit (Tan et al. 2012).

Based on various studies, tocotrienol have shown to exhibit antioxidant, antiproliferative, antiangiogenic and anti-inflammatory activities (Aggarwal et al. 2010). In addition, tocotrienol also exhibit neuroprotective and cholesterol lowering activity by inhibiting the activity of HMG-CoA reductase (Das et al. 2008). It also possesses anti-cancer and anti-diabetic properties (Aggarwal et al. 2010).

The aim of this research was to determine the effects of palm tocotrienol on the glucocorticoid-induced osteoporotic bones. In this study, the number of osteoblasts and osteoclasts in osteoporotic bones were observed when the tocotrienols isomer of vitamin E, mainly derived from palm were used. Decalcified bone samples from either tibia or femur were used for the analysis of the histological parameters. Using specific biochemical markers, the activities of osteoblasts and osteoclasts in both variables were analyzed.

Materials and Methods

Animal and Treatment

All procedures were carried out in accordance with the institutional guidelines for animal research surgical procedures of the Universiti Kebangsaan Malaysia (UKM) Research and Animal Ethics Committee (UKMAEC).

Forty three-month-old male Sprague-Dawley rats weighing about 250-300 g were obtained from the Universiti Kebangsaan Malaysia (UKM) Animal Breeding Centre. The animals were randomly divided into five groups (n=8) comprising of baseline (Baseline), sham operated (Sham) and three experimental groups supplemented with either palm olein (Adrx+Dexa), α-tocopherol (Adrx+Dexa+ATF) or palm tocotrienol (Adrx+Dexa+PTT). Adrenalectomy was performed on the Adrx+Dexa, Adrx+Dexa+ATF and Adrx+Dexa+PTT groups two days after receiving the animals. The rats were anaesthetized before the dorsal midline and bilateral flank muscle incisions were made. The adrenal glands were identified and the vessels were ligated to avoid bleeding before the removal of the glands. The incisions were sutured and the wounds were cleaned. Poviderm© cream was applied to the wound daily for five

days to prevent infection and also to aid in wound healing. The sham operated rats underwent the similar procedure except that the adrenal gland was left *in-situ*.

Two weeks post-adrenalectomy procedure, the rats were given 120 μg/kg dose of dexamethasone through intramuscular injection and followed with the administration of the following supplements; Adrx+Dexa+ATF received 60 mg/kg/day of α-tocopherol and Adrx+Dexa+PTT received 60 mg/kg/day of palm tocotrienol. The positive control group, Adrx+Dexa group was also given intramuscular dexamethasone and received orally 0.05 mL/100 g/day of palm olein. Palm olein is the vehicle used to deliver α-tocopherol and palm tocotrienol. All supplements were delivered via gastric gavage for six days a week for two months. The palm tocotrienol used in this study was provided by Sime Darby Bhd.

The animals were placed in clean cages under 12 h light and dark cycles. They were fed with rat pellets (Gold Coin, Malaysia) *ad libitium*. The adrenalectomized rats were provided with normal saline to drink *ad libitium* to replace the salt loss due to mineralocorticoid deficiency post-adrenalectomy, while the Sham group was given tap water. After two months of supplementations, the animals were euthanized and their tibia or femur was extracted.

SAMPLE COLLECTION

Blood were taken before the commencement and after the completion of the treatment. They were centrifuged at 3000 rpm at 4°C for 15 min and the serum was kept in aliquots at -80°C until analyzed.

Both tibia and femur bones from each rats were cleaned and fixed using periodate-lysine-paraformaldehyde for 24 h at 4°C. This is followed with washing using three solutions, 0.01M PBS containing 5% glycerol, 0.01M PBS containing 10% glycerol and 0.01M PBS containing 15% glycerol for 12 h at 4°C to decalcify the tissue. The specimens were further decalcified using EDTA-G solution for 10 to 14 days at 4°C. This solution was replaced every 5 days and the progression of decalcification was monitored every 24 h by micro X-ray.

Once decalcified, the reagent was washed several times using specific concentration of solutions, of 15% sucrose and 15% glycerol in PBS, 20% sucrose and 10% glycerol in PBS, 20% sucrose and 5% glycerol in PBS, 20% sucrose in PBS, 10% sucrose in PBS, 5% sucrose in PBS and lastly100% PBS. The decalcified tissues was then dehydrated in a series of alcohol and embedded in paraffin using an automatic tissue processor.

SERUM BONE BIOMARKERS ANALYSIS

Serum bone biomarkers were analyzed by utilizing the ELISA method. For serum resorption markers, carboxy-terminal collagen cross links (CTX) were measured using the Cross Linked C-Telopeptide of Type 1 Collagen Kit (Uscn Lifescience Inc. Wuhan, China), whereas serum osteocalcin was analyzed using the ELISA kit (Immunodiagnostic Systems Limited, UK). The serum used was obtained prior to the rats being sacrificed.

RANKL/OPG RATIO GENE EXPRESSION MEASUREMENTS

A fraction of the trabecular bone tissue (~5 mg) was obtained from the distal part of left femurs and placed into a vial containing magnetic beads. One vial was used for each sample. A mixture of 300 μL of Homogenising Solution (provided in the QG Sample Preparation Kit for Tissue) and 3 μL of Proteinase K (provided in the QG Sample Preparation Kit for Tissue) were added into the vial. The vials were later placed into Cell Rupture equipment for 15-30 s for 2 cycles followed by a quick spin for 5 s. The vials were then incubated in a warm water bath at 65ºC for 60 min and spun at 13,000 rpm for 10 min at room temperature. The homogenate/supernatant was transferred into a microcentrifuge tube and stored at -80ºC until used. The kits used for this procedure were supplied by Affymatrix Thermo Fisher Scientific, USA.

TISSUE SECTIONING

The paraffin block of each group was trimmed using microtome to produce five samples with the thickness of 5 micrometers each. These samples were floated on a water bath at 48°C and collected under Poly-L-lysine coated glass slides. The slides were stained histochemically with Alkaline Phosphatase (ALP) and Hematoxylin and Eosin (H&E) to observe osteoblast and osteoclast population, respectively.

HISTOLOGICAL DEMONSTRATION OF ALP FOR OSTEOBLAST

ALP histochemistry was performed following a modified version of a previously described method by Miao et al. (2002). The tissue section was deparaffinised and hydrated with xylene, followed by a series of alcohol graded solution. The slides were soaked overnight in 1% magnesium chloride in 100 mm Trismaleate buffer (pH9.2). prior to incubation with ALP substrate solution (freshly prepared) which contain 100 mM Tris-maleate buffer (pH9.2), 0.2 mg/mL naphthol AS-MX phosphate and 0.5 mg/mL Fast Red TR for 2 h in room temperature. After washing the section tissue with distilled water, Vector methyl green nuclear were used to counterstain the sample for 20 s and mounted with Kaiser's glycerol jelly.

HISTOLOGICAL DEMONSTRATION USING H&E STAINING FOR OSTEOCLAST

Hematoxylin and Eosin (H&E) histology was performed using the standard protocol. The slides were deparaffinized and rehydrated by using graded alcohol to distilled water. After hydration process, the slides were stained in Tacha's CAT Hematoxylin for 1 min. The slides were then washed 4-5 times with tap water and fixed with Phosphate Buffer Saline (PBS) pH7.2 to 7.3 for 1 min. The slides were rinsed with three changes of distilled water before counterstained

in Alcoholic-Eosin for 1 min. The Alcoholic-Eosin reagent contains 50.0 mL 1.0% Eosin Y (mixture of 1.0 g Eosin Y and 100 mL distilled water), 5.0 mL 1.0% Phloxine B (0.5 g Phloxine B in 50.0 mL distilled water), 390.0 mL 95% Ethanol and 4.0 mL Acetic Acid, glacial. The slides were then dehydrated through a series of three changes of 95% Ethyl Alcohol (EtOH) and two changes of 100% EtOH for 1 min each. This is followed by three changes of Xylene for 1 min each to clear the slides before covering with coverslips.

DATA COLLECTION FOR HISTOLOGICAL SECTION

The total number of cells (osteoblasts and osteoclast) with intact nuclei were observed at ×200 magnification at five different areas per section. A double-blinded experiment was performed to calculate the total number of cells using Weibel point counting method prior to statistical analysis.

STATISTICAL ANALYSIS

The population of osteoblast and osteoclast from different treatment groups were compared using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 23 (IBM, Armonk, USA). Values were considered statistically significant at $p<0.05$. All data were presented as mean ± standard error of mean.

RESULTS AND DISCUSSION

SERUM BIOMARKER ANALYSIS

Continuous treatment of dexamethasone for two-months did not produce any significant changes to the serum osteocalcin level between Adrx+Dexa against Baseline and Sham groups. Supplementation of α-tocopherol or PTT also bears no effect on the level of osteocalcin (Figure 1(a)). In contrast, serum CTX level showed a significant increase in the Adrx+Dexa group compared to the Baseline and Sham group ($p<0.05$). Supplementation with α-tocopherol (Adrx+Dexa+ATF) and palm tocotrienol (Adrx+Dexa+PTT) significantly lowered the CTX level ($p<0.05$) compared to Adrx+Dex group. CTX level between Adrx+Dexa+TT and Sham groups were not significantly different (Figure 1(b)).

GENE EXPRESSION ANALYSIS

Osteocalcin gene expression, which is often used as a marker for bone formation process were found to be significantly lowered in Sham, Adrx+Dexa+ATF and Adrx+Dexa+PTT groups compared to Baseline and Adrx+Dexa groups (Figure 2(a)). However, two months treatment caused decrease in bone resorption related gene expression of RANKL/OPG Ratio in Sham group compared to Adrx+Dexa and Adrx+Dexa+ATF groups. Supplementation with palm tocotrienol caused no significant changes to the RANKL/OPG ratio (Figure 2(b)).

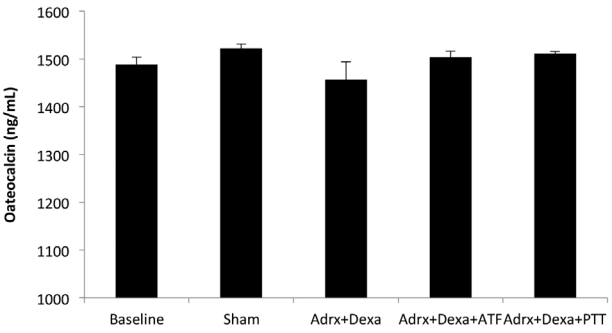

FIGURE 1(a). Serum osteocalcin. Data presented as mean + SEM. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)

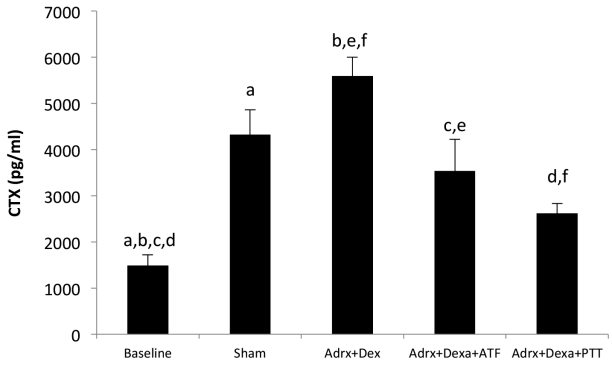

FIGURE 1(b). Serum Cross Linked C-Telopeptide of Type 1 Collagen (CTX). Data presented as mean + SEM. Same alphabets indicate significant difference between treatment groups at $p<0.05$. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)

EFFECTS OF THE SUPPLEMENTS TO THE PERCENTAGE OF SURFACE OSTEOBLAST

The percentage of osteoblast on the surface of normal bone and experimental rats using ALP staining are shown in Figure 3. Following the surgery and supplementation, no significant differences across the groups ($p>0.05$) were recorded. The surface osteoblasts are shown (arrowhead) in Photomicrograph 1 and are spread evenly across groups. These cells lie on the bone surface with spindle-shaped formation, indicating its inactive stage.

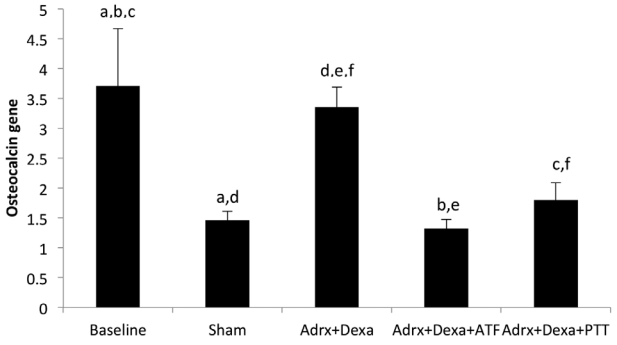

FIGURE 2(a). Bone formation gene expression. Data presented as mean + SEM. Same alphabets indicate significant difference between treatment groups at $p<0.05$. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)

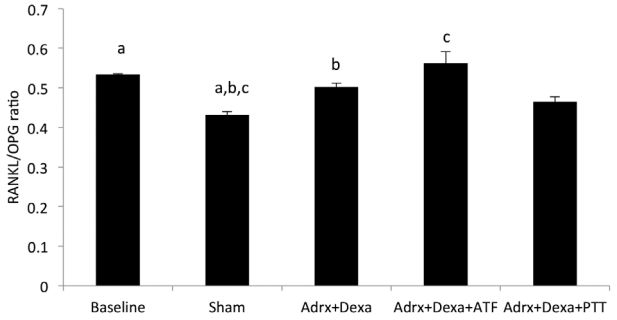

FIGURE 2(b). Bone resorption gene expression. Data presented as mean + SEM. Same alphabets indicate significant difference between treatment groups at $p<0.05$. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)

EFFECTS OF THE SUPPLEMENTS TO THE PERCENTAGE OF SURFACE OSTEOCLAST

The percentage of surface osteoclast on the bone of normal and supplemented rats using the conventional H&E is shown in Figure 4. There was a significant difference in percentage of surface osteoclast between the Sham and Adrx+Dexa group. The percentage of osteoclasts was also reduced significantly in the Adrx+Dexa+PTT group when compared to Adrx+Dexa group ($p<0.05$) and Sham group ($p<0.005$). However, α-tocopherol supplemented group and the baseline group showed no significant differences compared to the other groups. The surface osteoclasts are shown (arrows) in

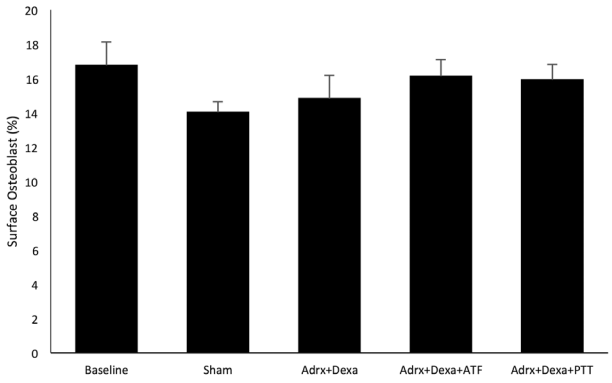

FIGURE 3. Effect of palm tocotrienol and α-tocopherol on surface osteoblast. Adrx+Dexa+PTT and Adrx+Dexa+ATF treated rats showed no differences against Adrx+Dexa, Sham or Baseline. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)

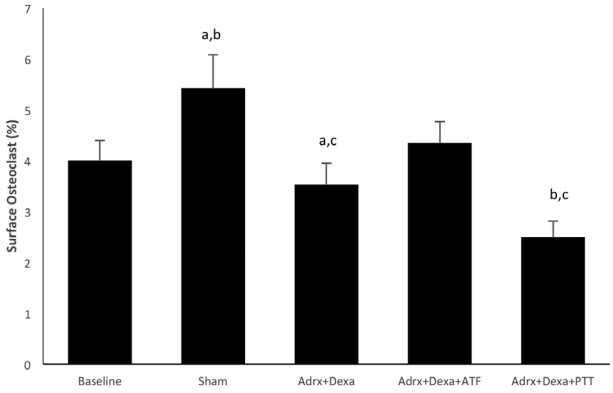

FIGURE 4. Effect of palm tocotrienol on the surface osteoclast. Adrx+Dexa+PTT was significantly reduced against Sham (b = $p<0.005$) and Adrx+Dexa (c = $p<0.05$), one-way ANOVA followed by post-hoc Tukey test. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)

Photomicrograph 2. These multinucleated cells were seen residing in the trabecula.

The effects of glucocorticoid in inducing osteoporosis has been long established. Glucocorticoid has been found to increase the oxidative stress in bone (Almeida et al. 2011). Removal of the adrenal gland and usage of glucocorticoid causing osteopenia in rats have been used as a model for secondary bone loss. The dosage of glucocorticoids used in our study was optimized by previous study which causes decrease in bone density

and biomechanical strength (Elvy Suhana et al. 2012). Glucocorticoids causes loss of cancellous bone with associated histomorphometric and biochemical decrease in bone formation, increase bone resorption and impaired bone strength. Glucocorticoids act on the stem cells differentiation which decrease the production of osteoblast (De Nijs 2008). Osteoblasts secrete RANKL, an osteoclast activator and also osteoprotegrin, a RANKL inhibitor. Glucocorticoids also induces the production of RANKL with subsequent increase in osteoclastic activity (Mitra 2011).

Two months treatment with glucocorticoids was not sufficient to increase serum osteocalcin levels as shown in our results. Osteoporosis would generally decrease calcium and phosphorus level leading to decreased hydroxyapatite crystal formation. Osteocalcin is a calcium dependent biomarker that has a strong affinity with bone matrix responsible for mineralization of bone. Its level is expected to increase in osteoporosis (Jagtap et al. 2011). Bone resorption marker of CTX was elevated in the Adrx+Dexa group and was reduced by the supplementation of palm tocotrienol. This effect was seen in a study using tocotrienol derived from annato fruit (Abdul-Majeed et al. 2012) but not observed in palm tocotrienol before (Soelaiman et al. 2012).

Our study also indicates that glucocorticoid-induced osteoporosis is characterized by an increase in osteoclast population but not osteoblast population. The changes of osteoclast population leads to an imbalanced resorption activity resulting in increased porosity of the bone. The percentage of osteoclast surface was significantly reduced ($p<0.05$) in the tocotrienol-treated group compared to controlled group and the controlled group compared to Sham group. This result showed the effects of tocotrienol in producing anti-oxidant effects of reducing the bone resorption by reducing the percentage of surface osteoclasts but not osteoblast. This is in agreement with the previous study that indicates vitamin E effect by regulating the osteoclasts fusion (Fujita et al. 2012).

In previous study, tocotrienol have shown to exhibit anti-oxidant, anti-proliferative, anti-angiogenic and anti-inflammatory activities (Aggarwal et al. 2010) which resulted in a prevention of bone loss and maintained bone microarchitecture. Tocotrienol supplementation prevent bone loss by increasing osteoblast survival through scavenging free radicals and protecting intercellular antioxidant defense system. Tocotrienol supplementation known to increase osteoblast proliferation while decreasing osteoclast proliferation by reducing the expression of pro-inflammatory cytokines that prevents IL-1 induced osteoclast formation (Nazrun et al. 2012). Besides that, it also down-regulates mevalonate pathway which increases osteoblast and decreases osteoclast proliferation. Tocotrienol supplementation also enhances expression of bone formation gene which conserves the bone density. Ha et al. (2011) found that tocotrienol specifically the alpha isomer, which can be found abundantly in palm tocotrienol has anti-bone resorptive properties by disrupting some of the key steps needed for osteoclastic bone resorption. This study also concluded that α-tocotrienol has the ability to inhibit the osteoclastic bone resorption by inhibiting nuclear factor–κB ligand (RANKL) expression in osteoblasts, suppressing RANKL action on osteoclast precursors, and reducing bone-resorbing activity of mature osteoclasts (Ha et al. 2011). The total cell number of osteoclast is not affected when its formation is inhibited by the α- and γ-tocotrienol isomers. However, inhibition of osteoclast is only exhibited by γ-tocotrienol and it was the most potent inhibitor of both osteoclast formation and activity (Brooks et al. 2011) .

Conclusion

This study demonstrated that the supplementation of palm tocotrienol has a beneficial effect in preventing osteoporosis by inhibiting osteoclastic activity as demonstrated by reducing its activity as shown by the reduced turnover marker CTX and histologically by reducing the number of osteoclast. This showed that palm-tocotrienol exert its anti-osteoporotic potential by inhibiting the osteoclastic activity but not through osteoblast augmentation. Further analysis is needed to support the use of palm tocotrienol as a supplementary diet especially in patients receiving long term glucocorticoid treatment.

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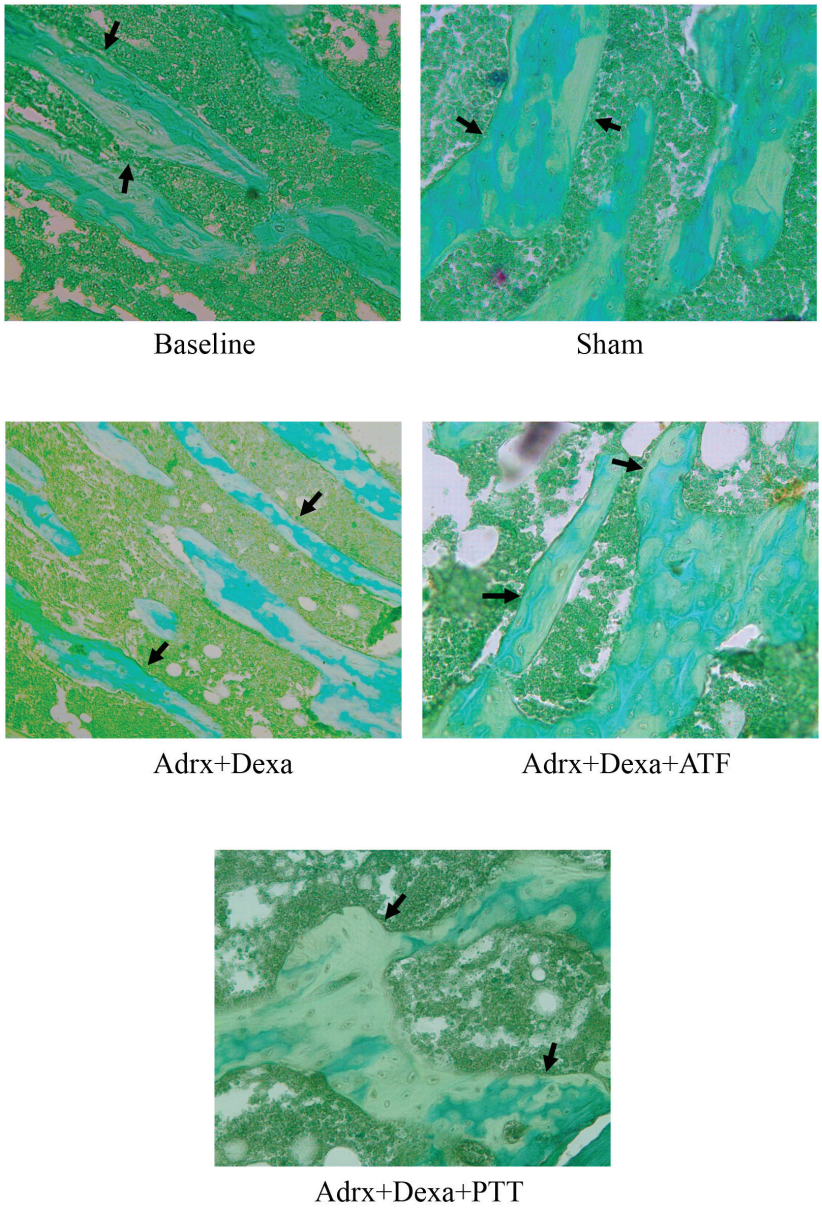
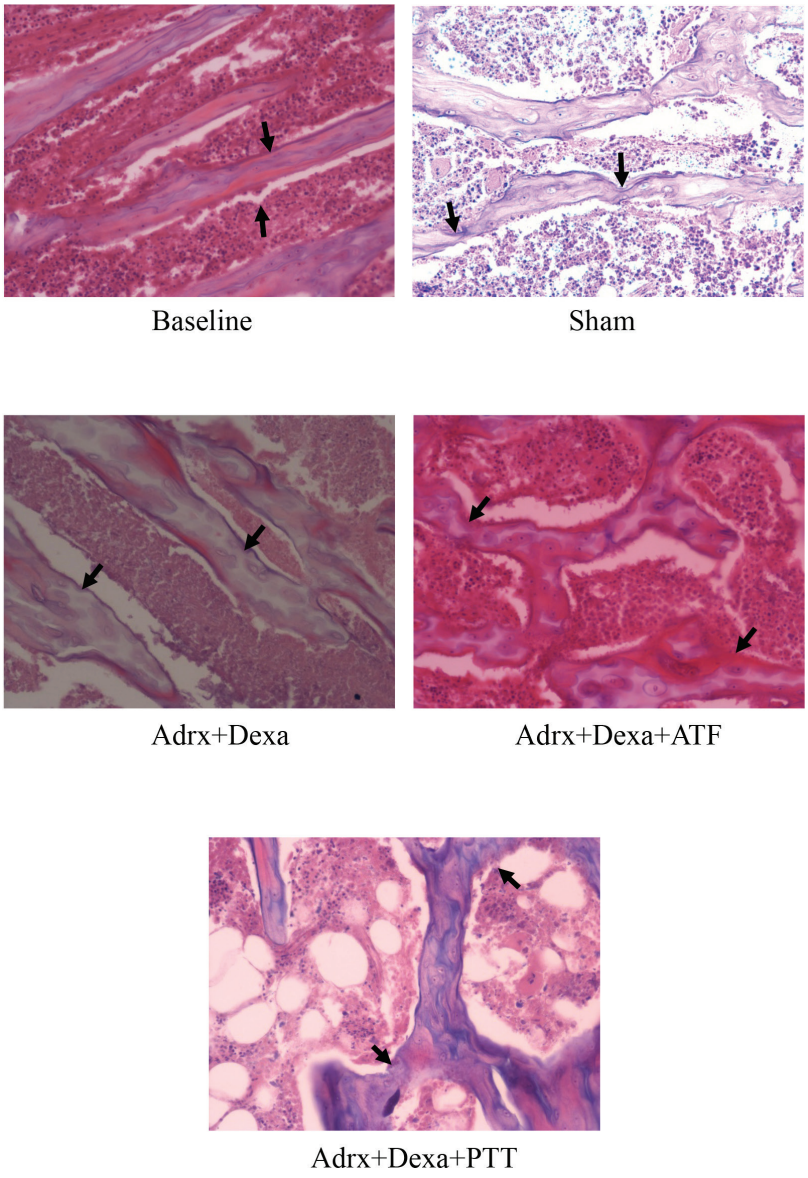


PHOTOMICROGRAPH 1. Bone histological section stained with ALP at 200× magnification. Surface osteoblasts (arrows) lie on the bone surface and mostly were in spindle-shaped form. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 µg/kg/day and oral palm tocotrienol 60 mg/kg/day)



PHOTOMICROGRAPH 2. Haematoxylin & eosin stain of decalcified bone at 200× magnification. Surface osteoclasts (arrows) observed on the bone surface and mostly residing in the trabeculae. Baseline = baseline control; Sham = sham operated control; Adrx+Dexa = adrenalectomized and given intramuscular dexamethasone 120 μg/kg/day; Adrx+Dexa+ATF = adrenalectomized and given intramuscular dexamethasone 120 μg/kg/day and α-tocopherol 60 mg/kg/day; Adrx+Dexa+PTT = adrenalectomized and given intramuscular dexamethasone 120 μg/kg/day and oral palm tocotrienol 60 mg/kg/day)