Anabolic Effects of Tocotrienol Isomers on Bone Formation in A Cancer-Induced Bone Pain Rat Model Using Procollagen Type I N-Terminal Propeptide as a Biomarker

Ofelia Binti Yahcob[1], Siti Nurfatimah Binti Mohd Shahpudin[2], Mohd Syahir Bin Mansor[3], Nur Izzati Binti Razali[1], Zaleha Binti Md Toha[4], Sharlina Binti Mohamad[1,5]*

[1]Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia

[2]Department of Biomedical Sciences, AMDI, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang,Malaysia

[3]Department of Biomedical Imaging, AMDI, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia

[4]Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang

[5]Breast Cancer Translational Research Program, AMDI, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia

*Correspondence:
Sharlina Binti Mohamad
sharlina@usm.my

Abstract – Cancer-induced bone pain (CIBP) is a significant clinical complication of bone metastases that severely compromises patient quality of life. Despite advances in contemporary therapies, the occurrence of adverse effects remains a significant limitation in current treatment strategies. Tocotrienols isoforms from vitamin E have demonstrated antioxidant and anti-inflammatory properties with potential roles in bone metabolism. Procollagen type 1 N-terminal propeptide (PINP) is a specific biomarker of osteoblastic activity and bone matrix synthesis. This study aimed to investigate the effect of tocotrienol isoforms on bone formation through PINP modulation in a rat model of CIBP. CIBP was induced by intrafemoral inoculation of breast cancer cells in female Sprague Dawley rats (n=40). The study was divided into five groups: (1) sham-operated, (2) cancer-induced untreated, (3) cancer-induced gamma tocotrienol-treated, (4) cancer-induced delta tocotrienol-treated, and (5) cancer-induced zoledronic acid-treated. Oral treatments were administered for 21 days. Blood samples were collected on day 21, and serum PINP levels were measured using the Rat PINP ELISA Kit. The findings suggest that tumour presence disrupts bone formation, while certain treatments may help counteract this effect. The γ- and δ-tocotrienols were significantly different from the Negative Control ($p < 0.05$), indicating a potential anabolic effect through stimulation of osteoblast activity. These results highlight the potential of tocotrienol isoforms as anabolic agents that may support bone health and complement existing antiresorptive therapies in managing cancer-induced bone pain.

Keywords – Breast cancer, CIBP, bone metastasis, tocotrienol isoform, PINP, bone formation

1 INTRODUCTION

Breast cancer is a major health burden in Malaysia, accounting for 8,371 (16.2%) of all cancer types and contributing to approximately 3,526 (11.1%) deaths per year [1]. In advanced breast cancer, 65–75% develop bone metastases.

CIBP is a debilitating condition associated with both increased bone resorption and suppressed bone formation. Consequently, bone formation markers such as PINP are reduced. This reduction is significant because PINP serves as a highly specific biomarker, directly reflecting osteoblastic activity and bone matrix synthesis, thereby indicating impaired bone formation [3]. Current analgesic treatments provide limited relief and are often associated with adverse effects. Although bone-targeted therapies are available, their applications remain restricted and do not fully address the problem. Vitamin E isoforms, particularly Gamma (γ)- and Delta (δ)- tocotrienol, are promising alternatives due to their high

antioxidant capacity, anti-inflammatory properties, anti-metastatic activity, and anti-resorptive properties [4]. Additionally, they possess bone-healing potential and have been shown to be safe, even at high doses. This study aimed to investigate the effect of tocotrienol isoforms on bone formation through modulation of PINP in a rat model of CIBP.

2 MATERIALS & METHODS

2.1 Cell Culture

The MDA-MB-231 breast cancer cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) containing high glucose (4.5 g/L) and stable glutamine, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin.

2.2 Animal

A total of 40 healthy female Sprague Dawley rats, aged 10-12 weeks and weighing 250-300g, were obtained from the Animal Research Section, Advanced Medical and Dental Institute, Universiti Sains Malaysia. The experimental procedure was approved by the USM Institutional Animal Care and Use Committee (USM IACUC) (USM/AICUC/2021/ (131) (1168) and was carried out in accordance with the standards for the care and use of laboratory animals. The rats were randomly assigned into five groups: (1) sham-operated, (2) cancer-induced untreated (negative control), (3) cancer-induced γ-tocotrienol-treated, (4) cancer- induced δ-tocotrienol-treated, and (5) cancer-induced zoledronic acid-treated (positive control). Each rat was marked on the tail for identification.

2.3 Cancer Inoculation and Treatment

CIBP was established by intra-femoral inoculation of 5 µL of MDA-MB-231 cells, at a concentration of 3×10^5 cells/mL using a Hamilton syringe. Treatment was delivered via oral gavage, with γ- and δ-tocotrienol administered daily at 1mg/kg for 20 consecutive days, and zoledronic acid administered at 0.1 mg/kg weekly. The sham group served as normal control, the cancer-induced untreated group as the negative control, the tocotrienol-treated groups as the experimental treatments, and the zoledronic acid-treated group as the positive control.

2.4 Blood Withdrawal

Blood was collected through cardiac puncture at the end of the 21-day experimental period. The whole blood obtained was allowed to clot at room temperature for one hour and subsequently centrifuged at 1,000 rpm for 20 min. The supernatant was carefully aspirated and used for the assay.

2.5 ELISA Assay

Serum PINP levels were measured using Rat PINP ELISA Kit (Elabscience, USA) in accordance with the protocol of the manufacturer. Absorbance was read at 450 nm ± 2 nm using a microplate reader.

2.6 Data Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 27. Group comparisons were performed with one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. Data are presented as mean ± standard error of the mean (SEM), and a p-value of < 0.05 was considered statistically significant.

3 RESULT & DISCUSSION

Our findings showed that the Negative Control group had the highest mean PINP concentration (2.79 ng/mL), which suggests significantly elevated bone turnover and collagen synthesis. The elevated PINP levels in this group reflect increased osteoblastic activity linked to tumour-induced bone remodelling. The increase in PINP indicates an attempt of osteoblasts to promote new bone formation in response to osteolytic damage caused by cancer invasion. Such a phenomenon is a hallmark of disrupted bone remodelling during metastatic progression. This observation is corroborated by Xu and Tang (2023), who noted a connection between PINP levels and the development of bone metastasis in breast cancer, highlighting that the combination of BSAP and TRACP-5b enhances diagnostic precision [5]. Furthermore, the current findings align with those of Baharuddin et al. (2024), who also reported elevated PINP levels associated with cancer-induced bone damage [6].

In contrast, the Sham group demonstrated the lowest PINP concentrations, reflecting the normal baseline bone turnover of healthy rats. Zoledronic acid (1.81 ng/mL) treatment did not show a significant difference compared to the Sham group but was significantly different from the Negative

Control group ($p<0.05$), indicating its effectiveness as a current therapeutic option. A Bayesian NMA study by Lorange et al. (2023) has reported that zoledronic acid significantly reduced new skeletal-related events (SREs), prolonged the time to SRE occurrence, and alleviated pain [7].

However, according to Sun et al. (2024), although zoledronic acid is clinically effective, its use is associated with a higher incidence of adverse events than placebo, emphasizing the need for careful monitoring and preventive strategies in clinical practice [8]. In this study, γ-tocotrienol (2.15 ng/mL) and δ- tocotrienol (1.96 ng/mL) were significantly different from the Negative Control ($p < 0.05$). The findings suggest that tocotrienol supplementation altered bone metabolism markers in the cancer induced model. These results indicate a potential anabolic effect through stimulation of osteoblast activity. However, limitations of this study include reliance on a single bone formation marker (PINP) and the use of an animal model that may not fully reflect human bone metastasis. Moreover, functional bone outcomes warrant further investigation in long-term and clinically oriented studies.

4 CONCLUSION

In conclusion, although zoledronic acid is primarily recognized for its antiresorptive effects, our findings indicate that γ- and δ-tocotrienol also contribute to the preservation of bone formation. Collectively, these results underscore the therapeutic potential of tocotrienol isoforms as anabolic agents that may enhance bone health and serve as complementary interventions alongside current antiresorptive therapies in the management of cancer-induced bone pain.

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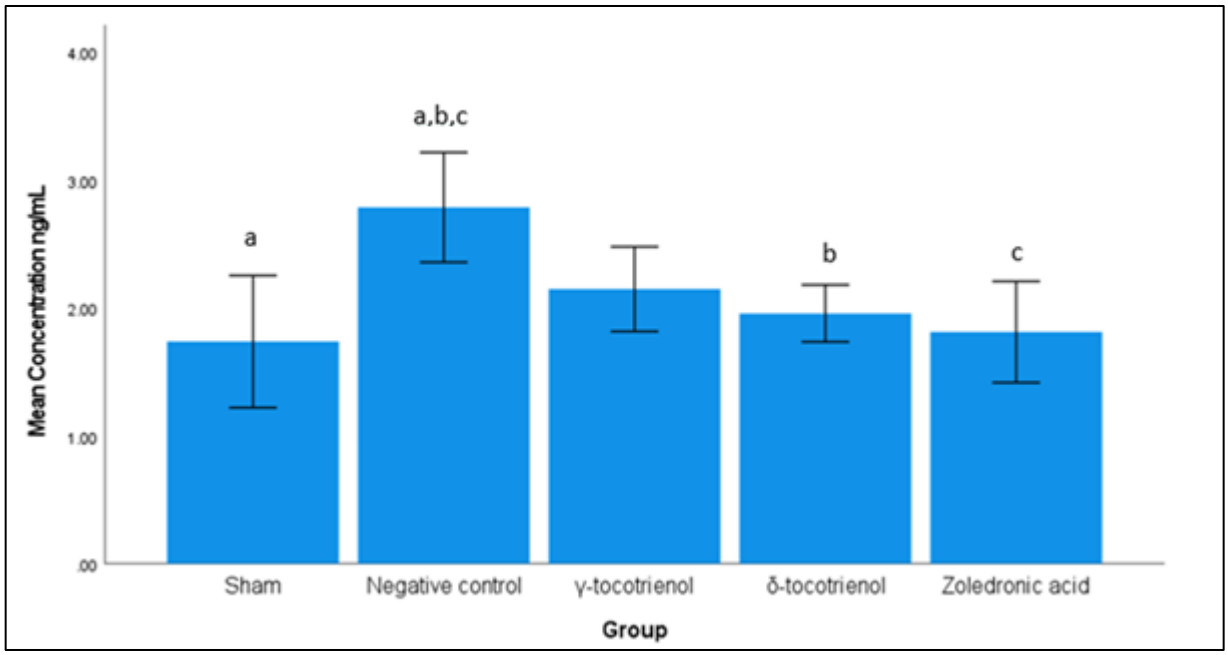

Figure 1. Mean serum Procollagen Type I N-terminal Propeptide (PINP) concentration across experimental groups. Bar graph showing the mean concentration of PINP (ng/mL) in Sham, Negative Control, γ-tocotrienol, δ-tocotrienol, and Zoledronic acid treatment groups. Data are presented as mean ± 95% confidence interval (CI). The Negative Control group exhibited the highest PINP concentration, indicating elevated bone turnover, whereas both treatment groups and the positive control group (γ- and δ- tocotrienol, and Zoledronic acid) demonstrated lower levels, suggesting attenuation of excessive bone formation.