Osteoprotective Effects of Virgin Coconut Oil and Tocotrienol-Rich Fraction on Biomechanical Bone Strength Parameter

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Objective: The study aimed to determine the bone effect of combined therapy of virgin coconut oil (VCO) and palm oil tocotrienol-rich fraction (TRF) on biomechanical bone strength parameters of the ovariectomised rat model fed with high fat diet and heated palm oil. **Materials and Methods:** Thirty-six right femora of female Sprague-Dawley rats were obtained from the previous study at Universiti Kebangsaan Malaysia (UKM). The bone samples were divided into six groups with six femora in each group: Sham-operated (SHAM), ovariectomised control (OVX), ovariectomised and given Premarin 64.5µg/kg (OVX+P), ovariectomised and given VCO 1.43ml/kg (OVX+V), ovariectomised and given TRF 30mg/kg (OVX+T) and ovariectomised and given combined therapy of VCO and TRF (OVX+VT); for 24 weeks of treatment. The right femora were wrapped completely in Phosphate Buffered Solution (PBS)-soaked gauze and aluminum foil and kept in the freezer (-80ºC). In the present study, the right femora were thawed at room temperature (25ºC) for two hours. The femora were analyzed for three-point bending test using Shimadzu, AG-X 500N machine controlled by proprietary software (Trapezium X Version 1.00, Shimadzu). **Results:** The data was tested for normality using the Kolmogorov-Smirnov test. The results showed that the data were normally distributed. The statistical tests used were the analysis of variance (ANOVA), followed by Tukey's HSD test. The Young modulus value of OVX+VT group was significantly higher than OVX+V and OVX+T groups (p<0.05). **Conclusion:** Combined supplementation of VCO and TRF have offered better bone protective effects than single supplementation with VCO or TRF in preventing bone loss of osteoporotic rat model.

Keywords: Osteoporosis, ovariectomy, virgin coconut oil, tocotrienol, three-point bending

1. Introduction

Osteoporosis can be defined as progressive systemic disease characterized by low bone mass and loss of bone tissue with a consequent increased risk of fracture[1]. Post-menopausal women have higher tendency to get osteoporosis compared to men due to oestrogen deficiency which led to reduced protection against oxidative stress[2].

The most important treatment is oestrogen replacement therapy (ERT), the gold standard treatment for post-menopausal osteoporosis. Unfortunately, prolonged use of this treatment causes long term adverse effects such as breast cancer, coronary heart disease, stroke and dementia[3]. These findings led for looking to the herbal supplementation in treating postmenopausal osteoporosis.

Virgin coconut oil (VCO) is one of the natural antioxidant-rich diets that can prevent bone loss caused by osteoporosis[4]. The single dose of VCO given to the ovariectomised rats resulted in improved bone structure and prevented bone loss due to its high polyphenols components which exert antioxidant property[5].

Furthermore, the other antioxidant-rich diet is palm oil tocotrienol-rich fraction (TRF) which has a major source of the fat-soluble vitamin E. TRF supplementation in ovariectomised rats, could improve bone biomechanical strength when tested biomechanically[6].

The current study was designed to determine the effects of both VCO and TRF, individually and in combination, on bone biomechanical properties (load, displacement, stiffness, stress, strain and Young modulus) of the ovariectomised rat fed with high cholesterol diet and repeatedly-heated palm oil.

2. Materials and Methods

Femora were harvested from a previous study (Approval No. UKMAEC: FP/ANAT/2014/FAIZAH/16-JULY/599-JULY-2014-SUG-2015-NAR-CAT2) on Sprague-Dawley rats conducted at Universiti Kebangsaan Malaysia (UKM). The femora were obtained from six different groups of female Sprague-Dawley rats. Sham-operated group (SHAM) fed on rat chow diet whereas the ovariectomised (OVX) control group was given 15% of five times heated palm oil (5HPO) mixed with 2%

cholesterol. The ovariectomised-treated groups were given 64.5µg/kg of Premarin (OVX+P), 1.43 ml/kg of virgin coconut oil (OVX+V), 30mg/kg of TRF (OVX+T) and combination of 1.43 ml/kg of VCO and 30mg/kg of TRF (OVX+VT).

Each right femur was wrapped with gauze soaked in Phosphate Buffered Solution (PBS) and rewrapped with aluminum foil to keep moist at all times in -80ºC freezer until use. On the day of testing, the femora were allowed to thaw at room temperature for 2 hours.

In this study, biomechanical properties of the right femoral bones were conducted using three-point bending test using the Universal Testing machine (Model Shimadzu, AG-X 500N) controlled by proprietary software (Trapezium X Version 1.00, Shimadzu). The parameters of the biomechanical strength were divided into the extrinsic parameters (load, displacement and stiffness) and the intrinsic parameters (stress, strain and Young modulus).

The data analysis was performed using the Statistical Package for Social Sciences software (SPSS 25; Chicago, IL, USA). The data was tested for normality using the Kolmogorov-Smirnov test (n=<100) and showed normally distributed data. Thus, the statistical tests used were the analysis of variance (ANOVA), followed by Tukey's HSD test.

3. Results

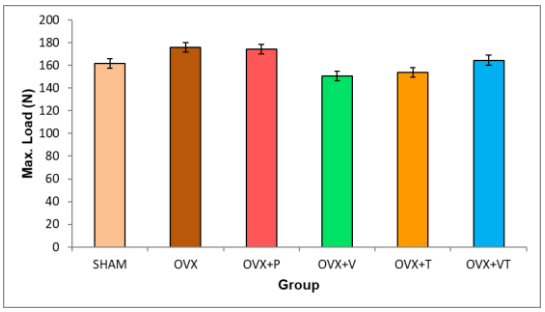

Figure 1: Maximum load value for all groups.

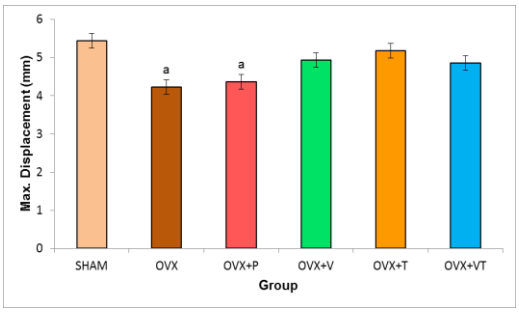

Figure 2: Maximum displacement value for all groups.
a* p<0.05 compared to the SHAM group

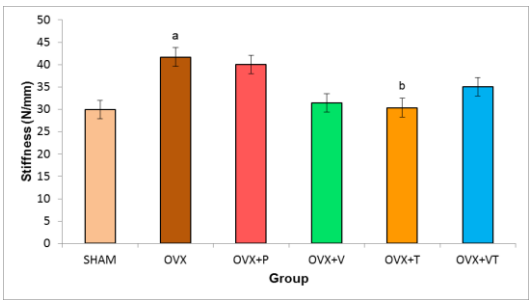

Figure 3: Maximum stiffness value for all groups.
a* p<0.05 compared to the SHAM group
b* p<0.05 compared to the OVX group

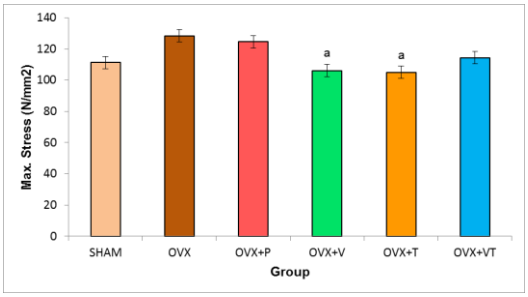

Figure 4: Maximum stress value for all groups.
a* p<0.05 compared to the OVX group

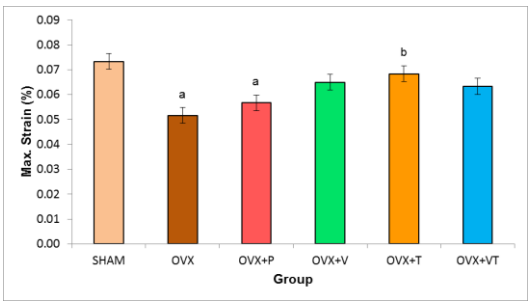

Figure 5: Maximum strain value for all groups.
a* p<0.05 compared to the SHAM group
b* p<0.05 compared to the OVX group

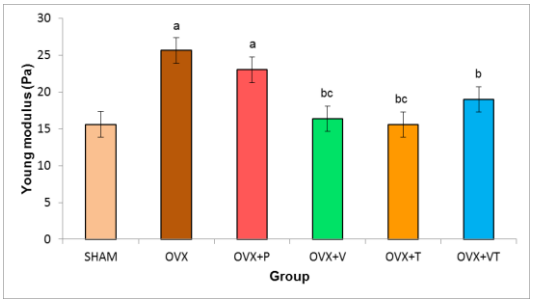

Figure 6: Young modulus value for all groups.
a* p<0.05 compared to the SHAM group
b* p<0.05 compared to the OVX group
c* p<0.05 compared to the OVX+P group

4. Discussion

The current study showed significant improvement in both extrinsic and intrinsic parameters. For extrinsic parameters, there was an increasing trend of displacement value between the treated groups (OVX+V, OVX+T and OVX+VT) compared to the OVX group. This showed that ovariectomised rats given these supplementations produced more ductile bones, hence they were harder to break. The result matched with previous studies which reported that decreased oestrogen level via ovariectomy led to decreased in cortical bone strength[2]. Nevertheless, other extrinsic parameters such as load and stiffness showed no statistical difference between all groups.

For intrinsic parameters, the OVX+T group showed more superior strain value than both OVX+V and OVX+VT groups. The result showed that supplementation with TRF offered the best protection against osteoporosis as shown by the significantly higher value than ovariectomised control group. Young modulus and maximum stress values of OVX+VT group was better than OVX+V and OVX+T groups. Thus, combined supplementation of VCO and TRF (OVX+VT group), have offered better bone protective effects than single supplementation with VCO or TRF.

5. Conclusion

Combined supplementation of VCO and TRF have offered better bone protective effects than single supplementation with VCO or TRF in preventing bone loss of osteoporotic rat model.

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7. References

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