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# CARROT *(DAUCUS CAROTA)* ROOTS EFFECTS ON BONE MINERAL CONTENT IN OVARIECTOMIZED RATS

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## ABSTRACT

The integrity of bone mineral density (BMD) is maintained through balance bone remodeling process which is mediated by coordinated activities of osteoblasts (bone formation cells) and osteoclasts (bone resorption cells), imbalance of this process where the activities of osteoclast out weight that of osteoblast bring about disease called osteoporosis and if this disease is as a result of estrogen deficiency is termed as postmenopausal osteoporosis often seen in aged women and ovariectomized (OVX) rats. This disease is regarded as public health problem with high morbidity and mortality among elderly women. The first effort to treat this disease was estrogen therapy however, its comes with several adverse effects. Phytonutrient has long been used to manage and treat chronic and aged related disease as they have less or no side effect, carrot roots (CRT) was used to evaluate its effect on bone mineral

## Introduction:

Postmenopausal osteoporosis is a disease of low bone mineral density (BMD) characterized by aberrant bone turnover with less bone formation and excessive bone resorption (Fouad-Elhady *et al.,* 2020). This disease is more common among elderly women and estrogen deprive ovariectomized (OVX) rats which are more predisposed to osteoporotic fractures as a result of imbalance of bone remodeling process mediated by bone cells of formation (Osteoblasts) and resorption (osteoclasts) (Li, *et al.,* 2019). Estrogen plays a crucial role in the preservation of bone homeostasis (Clauley, 2015), its deficiency increases the


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content in OVX rats. Twenty-four (24) female Wistar rats were OVX and six (6) were sham operated, the rats were divided into five groups (n=6): 1. sham rats, 2. OVX, 3. OVX + 200 mg/kg CRT, 4. OVX + 100 mg/kg CRT and 5. OVX + 5 mg/kg ALE and treated daily (orally) with CRT for six weeks. Some mineral composition of CRT was analyzed. The result obtained indicated that oral administration of 400 mg/kg CRT for 6 weeks significantly ($P<0.05$) elevated the content of femoral ash weight, calcium and phosphorus level and this may indicate the effect of CRT on bone minerals.

life of osteoclasts, it also decreases the longevity of osteoblasts, which function to build bone tissue (Lane, 2006).

Osteoblasts are known to synthesized biochemical metabolites that are involved in the calcification and mineralization of bone matrix which compose of calcium and phosphorus (Mada *et al.,* 2017 and Ross *et al.,* 1993).

Dietary intake of phytonutrient found in vegetables is evidently linked with bone health (Mackinnon *et al.,* 2011), as they are important sources of phytonutrient that have been shown to play vital role in the bone metabolism (Sanchez *et al.*, 2007). Higher consumption of fruits and vegetables has been correlated with a reduction in the risk for the development of postmenopausal osteoporosis (Bitto *et al*., 2009). Carrot (*Daucus carota*) is popularly known vegetable grown throughout the world which contains high level of β-carotene, fiber and many essential micronutrients and other functional ingredients (Sarfaraz *et al.,* 2016; Ndife, 2016). Oral intake of carrot juice has some beneficial physiological effects which include reduced oxidative DNA damage and increased levels of plasma antioxidants (Pool-Zobel *et al.,* 1998 and Owolade, *et al.,* 2017).

Hence, the present study sought to investigate the effects of carrot roots on mineral content in ovariectomized rats.

## Materials and Methods

### Reagents

The standard drug Alendronate was purchased from Sigma-Aldrich chemicals All other chemicals and reagents used in this study were of analytical grade and purchased from local suppliers.

## Experimental Animals

A total of 30 female wistar rats, weighing between 115 to 120 g were used. The animals were kept in well ventilated laboratory cages in the Animals House at room temperature and allowed to acclimatize to the surrounding for two weeks. They were provided access to standard rat chow diet and water *ad libitum*.

## Ovariectomy and Sham operation

Anaethesia was induced in the rats (n=24) by injecting each with ketamine hydrochloride (50 mg/kg body weight) and xylazine (12 mg/kg body weight) via the intraperitoneal route. Bilateral ovariectomy was carried out by ventral approach under aseptic condition following the method described by (Mada *et al.*, 2017). The remaining rats (n=6) underwent sham operation; this surgical procedure only differs from ovariectomy given that ovaries were identified but not removed. The rats were kept in separate cages for two weeks to recover and develop bone loss.

## Experimental Animal Grouping and Treatments

The OVX rats were randomly divided into four groups of six rats each while the sham operated rats made the fifth group (n=6). Group one Sham operated rats (Control), group two OVX untreated rats, group three OVXrats+200mg/kg CRT and group four OVXrats+400mg/kg CRT while group five OVX rats+5mg/kg body weight alendronate. The treatment of the groups described above commenced two weeks after ovariectomy and sham operation and continued for six weeks.

## Determination of Some Minerals Content in Carrot Roots

Calcium, magnesium, phosphorus, iron, copper and zinc, of the carrot root samples were determined following the method described by (Onwuka, 2018).

## Measurement of Femoral Bone Ash Weight, Calcium and Phosphorus Content

The right femora were cleaned of adherent tissues, dried in an incubator at 95 ºC for 12 h. The dried femora were weighed in a silica crucible and then transferred to a muffle furnace for ashing at 550ºC for 12 h. The crucible containing femora ash was taken out of muffle furnace and weighed again (ash weight). Bone ash-

calcium and phosphorus level in femora following the method described previously (AOAC 2005) using Atomic Absorption Spectrophotometer at 422.7 nm.

### Statistical Analysis

Data obtained were analyzed using SPSS version 20.1 and expressed as mean ± SEM mean value and analyzed using One-way Analysis of Variance (ANOVA). Statistically significant level was accepted at $P < 0.05$.

## Result

### Some mineral composition in CRT.

The result of some mineral composition of CRT are presented in Table 1. The result shows that the amount of calcium (37.58 ± 2.15 mg/100g), copper (0.03 ± 0.002 mg/100g), iron (0.41 ± 0.013 mg/100g), magnesium (11.21 ± 0.37 mg/100g), phosphorus (28.31 ± 3.14 mg/100g), zinc (0.19 ± 0.012 mg/100g).

**Table 1: Some mineral composition of CRT.**

| Parameters | (mg/100g) |
|---|---|
| Calcium | 37.58 ± 2.15 |
| Phosphorus | 28.31 ± 3.14 |
| Magnesium | 11.21 ± 0.37 |
| Copper | 0.03 ± 0.002 |
| Iron | 0.41 ± 0.013 |
| Zinc | 0.19 ± 0.012 |

### Effect of CRT on femur ash-weight, Calcium and Phosphorus level in OVX rats.

The result obtained demonstrated that femoral ash weight, calcium and phosphorus levels were significantly ($P<0.001$) reduced in OVX rats compared to control rats (Fig. 1a-c). Though, treatment of OVX rats with different concentrations of CRT significantly ($P<0.001$) ameliorated the reduction in these parameters in a dose-dependent fashion when compared to untreated OVX rats (Fig. 1a-c). Also administration of ALE to OVX rats indicated a significant ($P<0.001$) elevation of these indices more than CRT but lower than control rats.

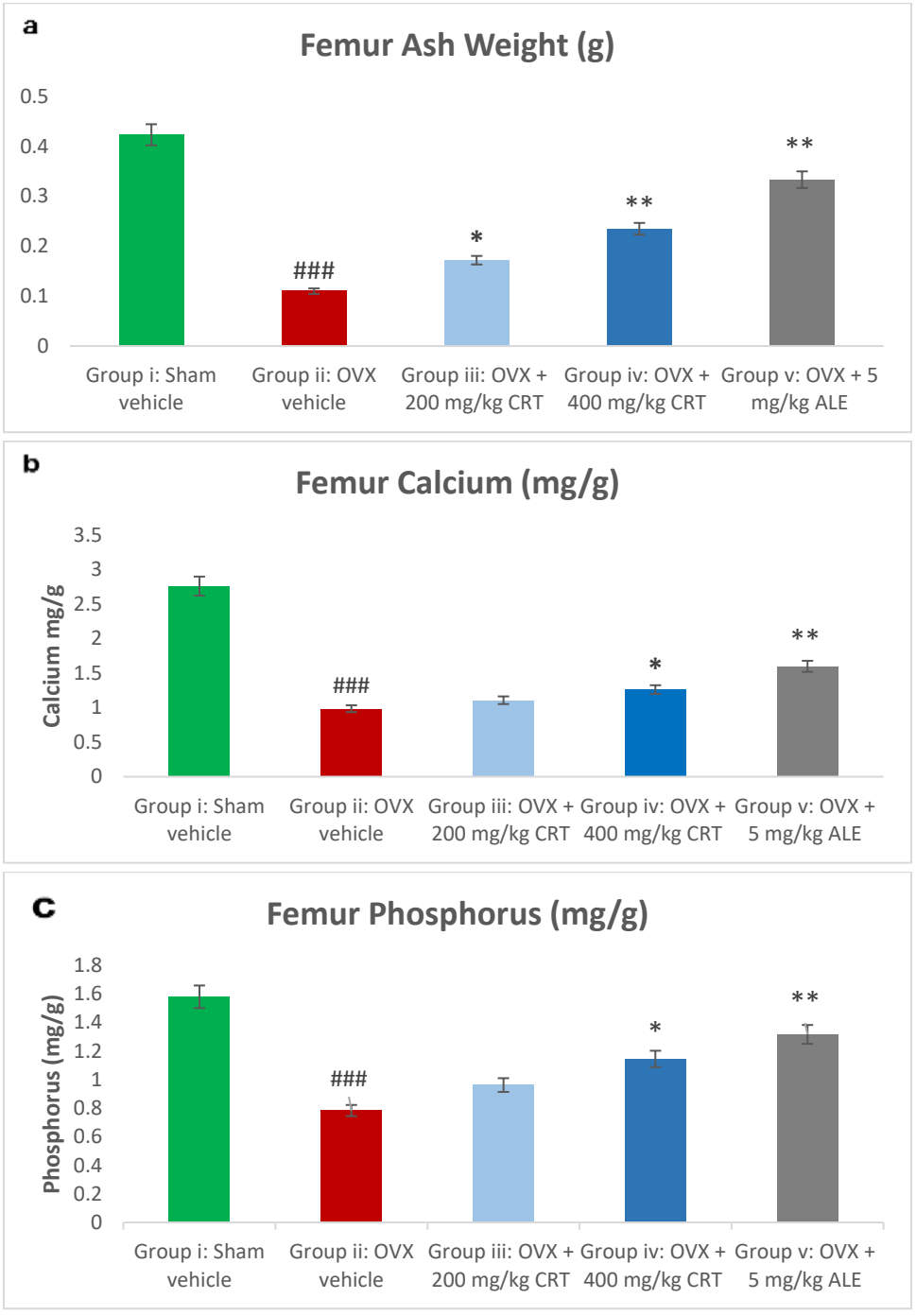


**Fig. 1: Effect of CRT on Femur ash-weight, Calcium and Phosphorus level in OVX rats.** (a) Femur ash-weight (b) Calcium level (c) Phosphorus level. Values are expressed a means ± SEM (n=6). $^{###}$ Significant ($P < 0.001$) when untreated OVX

group was compared to control group; *P < 0.05, **P < 0.01 Significant when CRT treated groups were compared with untreated OVX group. Control: Sham, OVX: Ovariectomized, CRT: Carrot, ALE: Alendronate.

## DISCUSSION

The present study does investigate the potential of carrot roots (CRT) on the femoral ash content, calcium and phosphorus in ovariectomized (OVX) rats. Phytonutrients found in several fruits and vegetables are link to have health benefits including bone health (Lennie 2001). Several studies associated phytochemicals in fruits and vegetables with reduction of chronic and age related diseases (Mohmmad *et al.*, 2017). CRT contains high amount of carotenoids; β-carotene being the highest in term of quantity as previously reported by Aurelia *et al.,* (2018), it also contains some level of flavonoids (Singh *et al.,* 2018) and several benefit of flavonoids on bone health has being reported (Liu *et al.,* 2015; Zhao *et al.,* 2019). Previous studies reported benefits of carrots consumption on human health (Deding *et al.,* 2020; Fujihara *et al.,* 2021) however, its positive effect on bone ash weight, calcium and phosphorus has not been evaluated to best on our knowledge. Ovariectomy operation is used to deprived rats with secretion of estrogen to mimic menopause condition and induce bone loss (Chalvon-Demersay *et al.,* 2017).

Bone strength result from the mineral salt in the osteoid matrix (hydroxyapatite) which constitute by calcium and phosphorus, Zaichick and Tzaphildou, (2002), reported that ratio of calcium and phosphorus may be used to diagnose bone disease. The data obtained in this study shows that the weight of femoral ash, calcium and phosphorus of OVX untreated group significantly reduced compared to Sham group, this result concur with previous studies that reported influence of ovariectomy in prevention of intestinal calcium re-absorption that result to decreased in these parameters (Elkomy *et al.,* 2015; Mada *et al.,* 2017) and also confirm the success of ovariectomy operation. CRT oral administration increased the femoral bone ash, calcium and phosphorus content dose dependently. This may be due to additional minerals content including calcium and phosphorus in which CRT contain considerable amount of these minerals as being analyzed in this study. However, low dose of CRT does not show significant difference in term of calcium and phosphorus compared to the OVX untreated rats group.

## Conclusion

In conclusion our data in the present study established evidence that CRT has exhibits its beneficial effects on bone mineral density potential, which effectively improve the content of femoral ash weight, calcium and phosphorus in estrogen deficient ovariectomized rats and could be act as an agent to reduced loss of bone mineral density.

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