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Osteoanabolic Effects of Carrot (Daucus carota) Roots on Ovariectomy-Induced Osteoporotic Rats

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Abstract

Estrogen depletion leads to bone loss (osteoporosis) mostly seen in postmenopausal women and ovariectomized rats. Osteoporosis is a silent disease with high mortality among elderly people. Several therapeutic treatments employed to correct/manage this disease were found to have specific adverse effect. The present study evaluated the effect of Carrot roots (CRT) on some bone remodeling biomarkers and anthropometrics parameters in ovariectomized (OVX) rats. Twenty-four (24) female Wistar rats weighing between 115 to 120 g, were Ovariectomized and six (6) were sham operated, the rats were divided into five groups, six rats per group: group one (sham control), group two (OVX untreated control), group three (OVX + 200 mg/kg body weight (b.w) CRT), group four (OVX + 400 mg/kg b.w CRT) and group five (OVX + 5 mg/kg b.w Alendronate) and treated daily for six weeks. The result elucidated that, oral administration of 200 and 400 mg/kg CRT for 6 weeks significantly ($P<0.05$) reduced over expression of remodeling biomarkers (Osteocalcine, Bone Alkaline Phosphate and RANKL), endometrial atrophy of the uterine histology, and elevated the serum estradiol level of the OVX rats ($P<0.05$) compared to OVX untreated rats. Moreover, oral administration of CRT for 6 weeks significantly reduced the loss of femur dry weight and femoral thickness of OVX rats. Hence this results suggested bone health benefit of carrot roots.

Keywords: *Carrot root, Osteoporosis, Ovariectomized rats, Bone Biomarkers, Osteoanabolic effect.*

Introduction

Bone is a dynamic and metabolically active tissue, it continually undergoes remodeling process mediated by the coordinated activities of bone resorbing cells (osteoclasts) and bone forming cells (osteoblasts). Imbalance between bone formation and resorption ultimately results in bone degeneration and a greater susceptibility to fractures (Song, *et al.,* 2017). Estrogen plays a crucial role in the preservation of bone homeostasis (Cauley, 2015). Its deficiency increases the life of osteoclasts, the cells that degrade bone tissue; it also decreases the longevity of osteoblasts, which function to build bone tissue (Lane, 2006). Post-menopausal women and estrogen-deficient ovariectomized (OVX) rats are more predisposed to developing osteoporotic fractures as a consequence of accelerated bone turnover from increased number and activity of osteoclasts (Li, *et al.,* 2019).

Estrogen replacement therapy (ERT) was the first line of action however, it comes with risks such as breast, ovarian, and endometrial cancers and therefore exceed its benefits in older women (Lee, *et al.,* 2011; Skjodt, *et al.,* 2019). The current antiresorptive therapies comprise the usage of selective estrogen receptor modulators, bisphosphonates, denosumab and strontium (Ettinger, *et al.,* 1999; Meunier, *et al.,* 2004; Murad, *et al.,* 2012; Bone, *et al.,* 2017), although these drugs show the desirable therapeutic effectiveness, semi-permanent use of them bring about many adverse events in the long run; like gastrointestinal problems, typical femoral subtrochanteric fractures, and some drug-specific infections (Qaseem, *et al.,* 2017; Lagari, *et al.,* 2018) .

Epidemiological evidence has indicated a link between dietary intake of fruits and vegetables and bone health (Mackinnon, *et al.,* 2011). Higher consumption of fruits and vegetables has also been correlated with a reduction in the risk for the development of postmenopausal osteoporosis Bitto, *et al.,* 2009). Carrot (*Daucus carota*) root (CRT) is a popular vegetables grown throughout the world. Biochemically it is loaded with carotenoids, phenolics compounds, vitamins C and E fiber and many essential micronutrients and functional ingredients (Alasalvar *et al.,* 2001; Sarfaraz *et al.,* 2016; Ndife, 2016). The present study was aimed to evaluate anabolic effect of CRT on estrogen deficient ovariectomized rats.

Materials and Methods

Reagents and Assay Kits

Rats RANKL, osteocalcin (OC) and estradiol ELISA kits were purchased from Wuhan Fine Biotech Co., Ltd. China. The standard drug Alendronate (ALE) was purchased from Sigma-Aldrich chemicals Co. Inc. (Milwaukee WI, USA). Rat bone alkaline phosphatase (BALP) kit was purchased from Agappe Diagnostics (Knonauerstrasse, Switzerland). All other chemicals and reagents used in this study were of analytical grade.

Experimental Animals

A total of 30 female wistar rats, weighing between 115 to 120 g were kept in well ventilated laboratory cages in the Animals House of the Department of Pharmacology, Ahmadu Bello University Zaria 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 (Mada *et al.,* 2017). Bilateral ovariectomy was carried out by ventral approach under aseptic condition following the method described by (Kaczmarczyk-Sedlak *et al.,* 2009). The remaining rats (n=6) underwent sham operation; this surgical procedure only differs from ovariectomy given that ovaries were identified but not removed. The sham group served as a positive control. 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. Experimental rats underwent mild anesthesia 24 hours after they received their last treatment and blood samples were collected into a tube via cardiac puncture. The blood samples were centrifuged to obtain the serum assayed for BALP activity, estradiol, osteocalcin and RANKL level.

Measurement of Percentage Body Weight Change

Weekly body weight of all the animals in each group were recorded using electronic weighing balance. Percentage body weight change of each animal group were calculated from their mean weekly weight and initial weight (Hefnawy, *et al.,* 2013).

Measurement uterine weight

After sacrifice of the rats, uteri were carefully dissected, weighed using an electronic weighing balance, and subsequently fixed in 10 % neutral buffered formalin for histological examination. About 5 µm pieces from the middle segment of each uterus were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax using the previously described protocol (Trivedi *et al.,* 2008). Representative transverse sections (5 µm) were stained with hematoxylin and eosin. Microscopic imaging of sections obtained was taken for microstructure, epithelial glands, and endometrial lumen using an Olympus inverted microscope (Waltham, MA, USA).

Measurement of serum estradiol level

Serum estradiol level was determined using rat estradiol ELISA kit (Wuhan Fine Biotech Co., Ltd. China) following the manufacturer's instruction. The principle of this assay kit was based on competitive ELISA detection method.

Measurement of serum Alkaline Phosphatase Activity

Serum alkaline phosphatase level was determined using BALP kit (Agappe Diagnostics Co., Ltd. Ernakulam, Kerala, India) following the manufacturer's instruction.

Measurement of serum osteocalcin level

Serum osteocalcin was determined using rat osteocalcin ELISA kit (Wuhan Fine Biotech Co., Ltd. China) following the manufacturer's protocol.

Measurement of serum RANKL level

Serum RANKL was measured using rat RANKL assay kit (Wuhan Fine Biotech Co., Ltd. China.) following the manufacturer's instructions.

Measurement of anthropometry parameters (length, thickness and weight)

The right femora were cleaned of adherent tissues, dried in an incubator at 95 ºC for 12 h. The dried femora length and thickness were measured using Digital Vernier Caliper (SAN, Japan) as described by (Mada *et al.,* 2017). The dried femora were weighed using electronic weighing balance.

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

Effect of Carrot roots on body weight gain

The result shows no significant ($P>0.05$) different in terms of the mean body weight of the experimental rats in each group at the beginning of the study (Table 1.) However, after six weeks of the study period, the body weight of untreated OVX rats were significantly ($P<0.05$) increased compared to control group. Interestingly, treatment of OVX rats with 200 and 400 mg/kg CRT significantly ($P<0.05$) suppressed the body weight gain in comparison to untreated OVX group. Moreover, as expected ALE (standard drug) significantly ($P<0.05$) decreases the body weight gain of the OVX rats almost similar to control group.

Table 1. Effect of Carrot roots on Percentage Body Weight Gain

Group	Control	OVX	OVX + 200mg/kg CRT	OVX + 400mg/kg CRT	OVX + 5mg/kg ALE
Initial Body Weight (g)	120.33± 3.86	120.67 ± 2.06	121.60 ± 5.99	119.25 ± 5.12	120.63 ± 2.05
Final Body Weight (g)	132.51 ± 2.16	159.33 ± 4.03	146.81 ± 11.72	137.31 ± 5.78	131.71 ± 5.25
Body Weight Gain (%)	**9.19**	**24.26**$^{###}$	**17.17***	**13.15****	**8.41*****

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

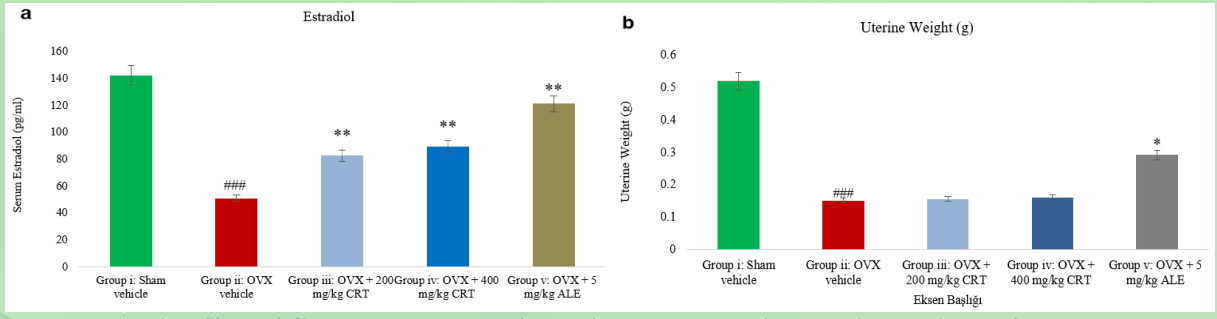

Fig. 1: Effect of Carrot roots on uterine weight and estradiol level in ovariectomized rats.

(a) Estradiol level (b) Uterine weight. 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. 1: SHAM, 2: Untreated OVX rats, 3: OVX rats+200mg/kg CRT, 4: OVX rats+400mg/kg CRT, 5: OVX rats+5mg/kg ALE

Effect of Carrot roots on uterine weight, Histology and estradiol Level and in OVX rats

Uterine weight and estradiol level in OVX rats were significantly ($P<0.05$) reduced compared to the control group (Fig. 1a & b). there was apparent atrophy of the endometrial lumen in OVX rats compared to sham-operated rats (Plate1 & 2). Treatment of OVX rats with different concentration of CRT significantly ($P<0.01$) elevates estrogen level ($P<0.001$) and reduced atrophy of the endometrial lumen of the OVX rats (Plate 3 &4) however, it does not reduce uterine weight loss when compared untreated OVX rats (Fig. 1a-b).

Effect of Carrot roots on Osteoanabolic (ALP and Osteocalcine) and bone-resorbing Markers (RANKL) in OVX rats

The effect of CRT on the level of osteoanabolic (ALP and Osteocalcin) and bone-resorbing markers in OVX rats were investigated. The data obtained shows that OVX-induced significant ($P<0.001$) elevation of these biomarkers and altered bone-remodeling in OVX rats compared to control group (Fig. 2a-c). Interestingly, when OVX rats were treated with 200 and 400 mg/kg doses of CRT, significant ($P<0.001$) reduction of these biomarkers of bone remodeling were observed in comparison to untreated OVX group (Fig. 2a-c).

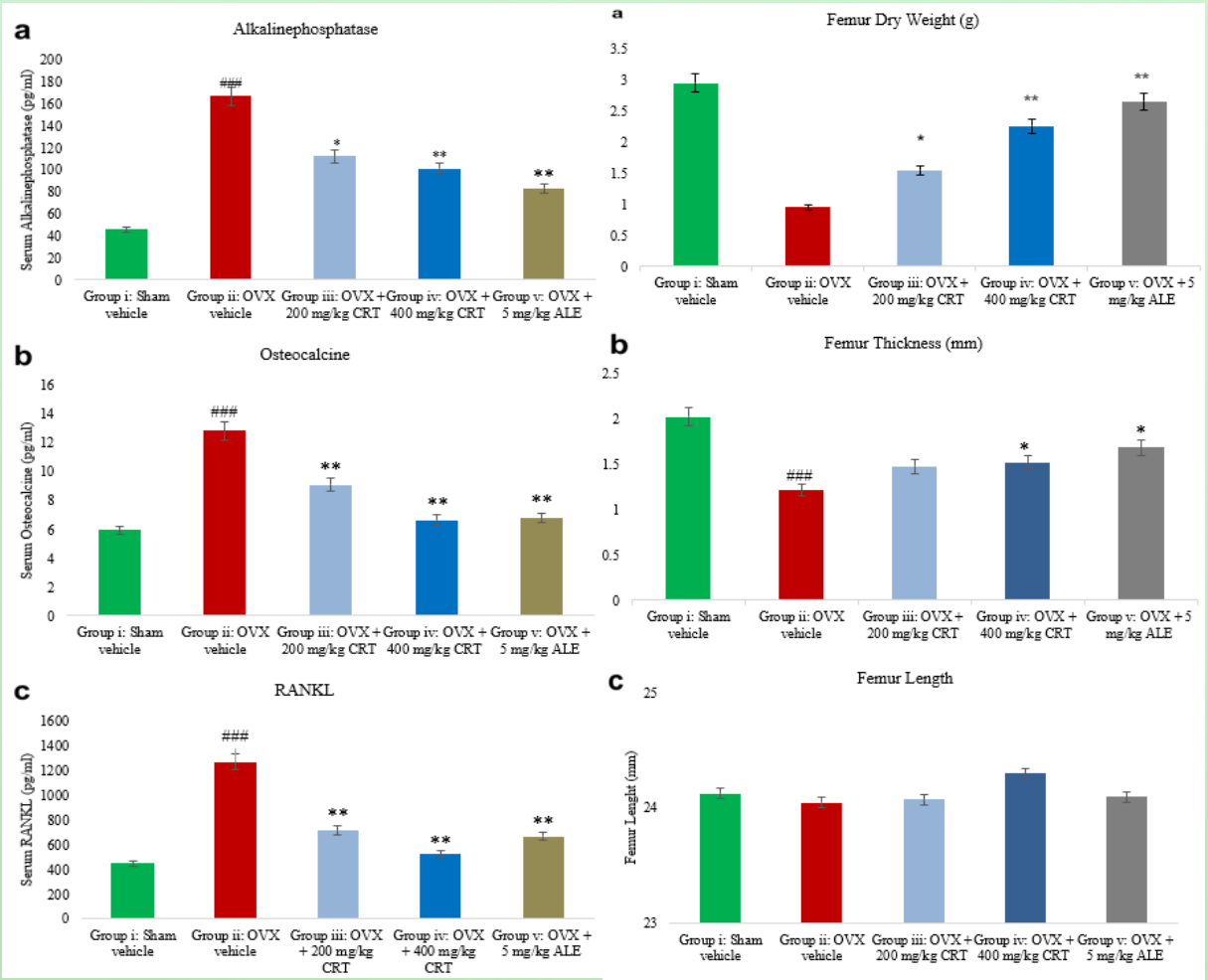

Fig. 2: Effect of carrot root on osteoanabolic and bone-resorbing markers in ovariectomized rats

(a) ALP activity (b) Osteocalcin level (c) RANKL 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 root, ALE: Alendronate.

Fig. 3: Effect of carrot root on anthropometric parameters in ovariectomized rats.

(a) Femur dry weight (b) Femur thickness (c) Femur length. 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 root, ALE: Alendronate

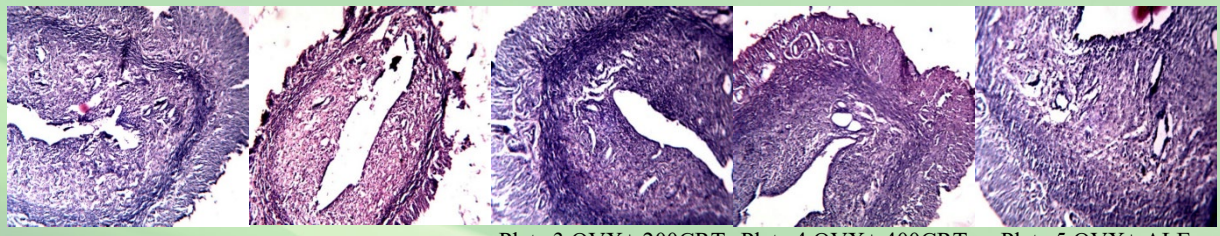

Plate 1 SHAM Group i | Plate 2 OVX Group ii | Plate 3 OVX+ 200CRT Group ii | Plate 4 OVX+ 400CRT Group iv | Plate 5 OVX+ ALE Group v

Effect of CRT on Femur anthropometric parameters in OVX rats

This study investigated the effect of CRT on anthropometric parameters (femur dry weight, thickness, and length). The data indicated the femur dry weight and femur thickness were significantly ($P<0.05$) altered in OVX rats compared to control rats (Fig. 3a & b). Interestingly, treatment of OVX rats with 200mg and 400mg of CRT significantly ($P<0.001$) mitigated the reduction of these anthropometric parameters in dose-dependent manner compared to untreated OVX rats. However, there was no significant ($P>0.05$) difference on femur length between OVX rats and control rats (Fig. 4c). Similarly, treatment of OVX rats with different doses of CRT did not showed any effect on femur length when compared to control rats (Fig.3c).

Discussion

The objective of the present study was to investigate the potential of carrot roots (CRT) on post-menopausal osteoporosis using ovariectomized (OVX) rats. Previous reports have linked consumption of fruit and vegetable to bone health (Mackinnon, *et al.,* 2011), this may be due to its nutrients including vitamins, minerals and other phytonutrients that are present in plants generally. CRT contains high amount of β-carotene (Aurelia, *et al.,* 2009). Previous studies reported the benefits of carrots consumption on human health (Deding, *et al.,* 2020; Fujihara, *et al.,* 2021) however, the use of carrot roots on bone health is lacking. Animal model established by ovariectomy is popularly known to be used for inducing rapid bone loss in animals to mimic physiological features of postmenopausal women by alteration of bone remodeling process (Chalvon-Demersay *et al.,* 2017) which associated with estrogen (E2) deficiency, weight gain and over expression of inflammatory cytokines (Fraser *et al.,* 2017).

The result of untreated OVX rats group in our study demonstrated increase in weight gain, reduction in serum β-estradiol level and uterine weight. The reduction of β-estradiol confirmed success of the ovariectomy surgery and also induction of menopause condition in the rats, this is in line with the several previous studies which stated that OVX is associated with body fat redistribution to the abdomen, weight gain, and decrease uterine size, attributed to decreased in estrogen level (Davis, *et al.,* 2012; Curtis *et al.,* 2018). Conversely, oral administration of CRT suppressed weight gain and enhances serum estradiol level in the OVX rats in a dose dependent manner hence, our data on weight gain concur with earlier result presented by (Fujihara, *et al.,* 2021) The results of uterine weight and histology in the present study revealed that CRT administration to OVX rats does not have any impact of reducing uterine weight loss of the OVX rats however, reduced endometrial atrophy of the uterus of the OVX rats dose dependently.

Preservation of bone quality is directly related to the balance of remodeling process, which must be maintained throughout life to spare bone integrity and its mass density (Vijayan *et al.,* 2009). Bone remodeling is regulated by hormones, enzymes and anti-inflammatory cytokines to maintained normal function of osteoclast and osteoblast activities (Kuo & Chen 2017), therefore these biochemical markers can be used to monitor and evaluate bone health or its diseases. OC, enzymatic activity of BALP, TNF, IL-6 and RANKL are among the effective tools to find out bone health or otherwise (Liu *et al.,* 2016). Recent study revealed that ovariectomy operation is associated with high elevation of serum BALP activity (Yang, *et al.,* 2019). Consistently, in this study we observed higher level of serum BALP activity and OC concentration in the OVX untreated group, this may further have indicated rapid bone turn over. Dose dependently CRT impacted positively upon these bone biomarkers, with the higher dose of CRT mitigated the elevation of both serum BALP and OC almost similar to the OVX group treated with the standard drug (Alendronate), this implies potential role of CRT in lowering bone turn over biomarker. More so, at lower dose both APL and OC concentration were reduced significantly compared to the untreated OVX group. In addition, over expression of RANKL due to estrogen deficiency by osteoblast which binds its receptor RANK on surface of osteoclast to stimulate the cells to mature, differentiate and increase bone resorption activity (Callaway & Jiang 2015; Kuo & Chen 2017), the data showed positive effect of CRT regulating excessive RANKL over-expression in dose depended manner, more so positive impact of carotenoids has been reported by several studies on regulating excessive expression of RANKL (Maggio, *et al.,* 2006; Yang, *et al.,* 2008; Sahni, *et al.,* 2009).

Furthermore, anthropometry parameters (femur dry weight, thickness and length) were investigated in the present study, femurs are reported to be more prone to deterioration compared to other bones (Hodgson *et al.,* 2008) moreover, various studies reported bone loss of some anthropometry parameters attributed to rapid bone demineralization (Khajuria, *et al.,* 2015; Samarghandian, *et al.,* 2016; Dabbaghmanesh, *et al.,* 2017). Treatment of OVX rats with CRT impact positively on femur weight and thickness compared to OVX untreated rats group, this may probably confirm the effect of CRT on bone turnover makers and further concur with previous studies on effect of carotenoids on post-menopausal osteoporosis (Yang, *et al.,* 2008; Sahni, *et al.,* 2009) this evidently showed potential of CRT to minimize bone loss. However, the result of femur length obtained in our study showed that CRT does not have any effect.

Conclusion

Conclusively, the findings in this study established evidence that CRT exhibits anti-bone resorption activities potential, which effectively improve level of estrogen, suppress RANKL over expression, restored ALP and OC bone turnover markers in OVX rats. Moreover, the present findings demonstrated beneficial impact of CRT on bone femur thickness and weight in OVX rats. More so, the result of the study supports the earlier epidemiological data regarding effect of CRT on obesity through reduction of weight gain induced by ovariectomy. Altogether, these finding suggested that CRT may be beneficial therapeutic agent against bone loss in OVX rats.

Acknowledgement
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Abbreviations

ALE: Alendronate, BALP: Bone alkaline phosphatase, CRT: Carrot roots, ERT: Estrogen replacement therapy, g: Gram, IL-6: Interleukin 6, Kg: Kilo gram, mg: Milligram, OC: Osteocalcine, OVX rats: Ovariectomized rats, OVX: Ovariectomy, RANKL: Receptor activator of NF k-β ligand, Sham group: Control group, TNF-α: Tumour necrosis factor alpha

References

Alasalvar, C., Grigor, J.M., Zhang, D, Quantick, P.C., & Shahidi, F. (2001). Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *Journal of Agric and Food Chemistry,* 49, 1410-1416.

Aurelia, S., Carmela, G., Leone, D., Rita, A. & Angelo, S. (2018). Phytochemical Analysis and Antioxidant Properties in Colored Tiggiano Carrots. *Agriculture*, 8, 102. doi:10.3390/agriculture8070102

Bitto, A., Burnett, B.P., Polito, F., Levy, R.M., Marini, H., Di Stefano, V., Irrera, N., Armbruster, M.A., Minutoli, L., Altavilla, D & Squadrito F. (2009). Genistein aglycone reverses glucocorticoid induced osteoporosis and increases bone breaking strength in rats: a comparative study with alendronate. *Britain Journal of Pharmacology* 156, 1287–1295.

Bone, H.G., Wagman, R.B., Brandi, M.L., Brown, J.P., Chapurlat, R., Cummings, S.R., Czerwinski, E., Fahrleitner-Pammer, A., Kendler, D.L., Lippuner, K., Reginster, J.Y., Roux, C., Malouf, J., Bradley, M.N., Daizadeh, N.S., Wang, A., Dakin, P., Pannacciulli, N., Dempster, D.W. & Papapoulos, S. (2017). Ten (10) years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. *Lancet Diabetes Endocrinology,* 5(7), 513–523.

Callaway, D.A. & Jiang J.X. (2015). Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. *Journal Bone Minerals Metabolism*, 33, 359-370.

Cauley, J.A. (2015). Estrogen and bone health in men and women. *Steroids,* 99, 11–15.

Chalvon-Demersay, T., Blachier, F., Tomé, D. & Blais, A. (2017). Animal models for the study of the relationships between diet and obesity: a focus on dietary protein and estrogen deficiency. *Frontiers in Nutrition*, 4, 5.

Curtis, K. S., McCracken, K., Espinosa, E., Ong, J., Daniel, J., Buck, D. J. & Davis, R. L. (2018). Temporal and site-specific changes in central neuroimmune factors during rapid weight gain after ovariectomy in rats. *Journal of Neurochemical Research,* 6, 18-25. https://doi.org/10.1007/s11064-018-2596-6

Dabbaghmanesh, M.H., Noorafshan, A., Talezadeh, P., Tanideh, N., Koohpeyma, F., Iraji A., Bakhshayeshkaram, M. & Montazeri, N.N. (2017). Stereological investigation of the effect of *Elaeagnus angustifolia* hydro alcoholic extract on osteoporosis in ovariectomized rats. *Avicenna Journal of Phytomedicine*, 7(3), 261-274.

Davis, S.R., Castelo, C.B., Chedraui, P., Lumsden, M.A., Nappi, R.E. & Shah, D. (2012). Understanding weight gain at menopause. *Climacteric,* 15, 419–429.

Deding, U., Baatrup, G., Christensen, L.P., & Larsen, M.K. (2020). Carrot Intake and Colorectal Cancer: A Prospective Cohort Study of 57,053 Danes. *Nutrient,* 12, 332. doi:10.3390/nu12020332.

Ettinger, B., Black, D.M., Mitlak, B.H., Knickerbocker, R.K., Nickelsen, T., Genant, H.K., Christiansen, C., Delmas, P.D., Zanchetta, J.R., Stakkestad, J., Gluer, C.C., Krueger, K., Cohen, F.J., Eckert, S., Ensrud, K.E., Avioli, L.V., Lips, P. & Cummings, S.R. (1999). Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial, Multiple Outcomes of Raloxifene Evaluation (MORE) investigators. *Journal of American Medical Association,* 282(7), 637–645.

Fraser L. C., Naiomy D. R., Shelby A., Hayley B., Daniel S., Jason N. B., Laura R. M. & Narayanan P. (2017). Temporal and regional intestinal changes in permeability, tight junction, and cytokine gene expression following ovariectomy-induced estrogen deficiency. *Journal Physiological Report,* 5(9). https://doi.org/10.14814/phy2.13263

Fujihara, K., Nogawa, S., Saito, K., Horikawa, C., Takeda, Y., Cho, K., Ishiguro, H., Kodama, S., Nakagawa, Y. & Matsuzaka, T. (2021). Carrot Consumption Frequency Associated with Reduced BMI and Obesity through the SNP Intermediary rs4445711. *Nutrients* 13, 3478. https://doi.org/10.3390/nu13103478

Hefnawy, T.M., Hefnawy, M. & Fawzy, R. (2013). Protective effects of Lactuca sativa ethanolic extract on carbon tetrachloride induced oxidative damage in rats. *Asian Pacific Journal of Tropical Disease*. 3(4), 277-285

Hodgson, J.M., Devine, A., Burke, V., Dick, I. M. & Prince, R. L. (2008). Chocolate consumption and bone density in older women. *American Journal of Clinical Nutrition,* 87(1), 175–180.

Kaczmarczyk-Sedlak I, Folwarczna J, Trzeciak HI. (2009). Thalidomide affects the skeletal system of ovariectomized rats. *Pharmacology Report*, 61, 529-538.

Khajuria, D.K., Razdan, R. & Mahapatra, D.R. (2015). Additive effects of zoledronic acid and propranolol on bone density and biochemical markers of bone turnover in osteopenic ovariectomized rats. *Rev Brasileira de Rheumatology*, 55(2), 103–112.

Kuo, T.R. & Chen, C.H. (2017). Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomarker Research,* 5, 18.

Lagari, V., Gavcovich, T. & Levis, S. (2018). The good and the bad about the 2017 American college of physicians' osteoporosis guidelines. *Clinical Therapeutics,* 40(1): 168–176.

Lane, N.E. (2006). Epidemiology, etiology, and diagnosis of osteoporosis. *American Journal of Obstetrics and Gynecology.* 194(2), 3-11.

Lee, S.H., Na, J., Doo-Jin, P., Deog-Yoon K., Ill-Min, C. & Yongsoon P. (2011). Consumption of legumes improves certain bone markers in ovariectomized rats. *Nutrition,* 31, 397–403.

Li, Z., Yuan, G., Lin, X., Liu, Q., Xu, J., Lian, Z., Song, F., Zheng, J., Xie, D., Chen, L., Wang, X., Feng, H., Zhou, M., & Yao, G. (2019). Dehydrocostus lactone (DHC) suppresses estrogen deficiency-induced osteoporosis. *Journal of Biochemical Pharmacology,* 163, 279–289.

Liu, L. & Webster, T.J. (2016). In situ sensor advancements for osteoporosis prevention, diagnosis, and treatment. *Current Osteoporosis Report,* 14, 386–395.

Mackinnon, E.S., Rao, A.V., Josse, R.G & Rao, L.G. (2011). Supplementation with the antioxidant lycopene significantly decreases oxidative stress parameters and the bone resorption marker N-telopeptide of type I collagen in postmenopausal women. *Osteoporosis International,* 22, 1091–1101.

Mada, S.B., Reddi, S., Kumar, N., Kumar, R., Kapila, S., Kapila, R., Trivedi, R., Karvande, A & Ahmad, N. (2017). Antioxidative peptide from milk exhibits anti-osteopenic effects through inhibition of oxidative damage and bone-resorbing cytokines in ovariectomized rats. *Nutrition,* 43, 21-31

Maggio, D., Polidori, M. C., Barabani, M., Tufi, A., Ruggiero, C., Cecchetti, R., Aisa, M. C., Stahlb, W. & Cherubini, A. (2006). Low levels of carotenoids and retinol in evolutional osteoporosis. *Bone,* 38, 244–248.

Meunier, P.J., Roux, C., Seeman, E., Ortolani, S., Badurski, J.E., Spector, T.D., Cannata, J., Balogh, A., Lemmel, E.M., Pors-Nielsen S., Rizzoli, R., Genant, H.K. & Reginster, J.Y. (2004). The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *New England Journal Medicine,* 350(5), 459–468.

Murad, M.H., Drake, M.T., Mullan, R.J. Mauck, K.F., Stuart, L.M., Lane, M. A., Abu Elnour, N.O., Erwin, P.J., Hazem, A., Puhan, M.A. Li, T., & Montori, V.M. (2012). Comparative effectiveness of drug treatments to prevent fragility fractures: asystematic review and network meta-analysis. *Journal of Clinical Endocrinology and Metabolism,* 97(6), 1871–1880.

Ndife J. (2016). Functional Foods Basics, Ingredients and Applications. *Amotes Link Services and Publishers.* 4. ISNB-978-978-54127-2-4

Qaseem, A., Forciea, M.A., McLean, R.M. & Denberg, T.D. (2017). Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American college of physicians, *Annals of Internal Medicine,* 166(11), 818–839.

Sahni, S., Hannan, M.T., Blumberg, J., Cupples, L.A., Kiel, D.K. & Tucker, L.K. (2009). Protective Effect of Total Carotenoid and Lycopene Intake on the Risk of Hip Fracture: A 17 Year Follow-Up from the Framingham Osteoporosis Study. *Journal of Bone and Mineral Research,* Volume 24, Number 6,

Samarghandian, S, Azimin-Nezhad M, Samini, F. and Farkhondeh, T. (2016). Chrysin treatment improves diabetes and its complications in streptozotocin-induced diabetic rat. *Canadian Journal of Physiology and Pharmacology,* 94(4), 388-393.

Sarfaraz S, Farooq N, Ashraf N, Aslam A, & Sarwar G. (2016). Non Pharmacological Use of Daucus Carota Juice (Carrot Juice) as Dietary Intervention in Reducing Hypertension. Enz Eng 5, 147. doi:10.4172/2329-6674.1000147.

Skjodt, M.K., Frost, M., & Abrahamsen, B. (2019). Side effects of drugs for osteoporosis and metastatic bone disease. *British Journal of Clinical. Pharmacology,* 85(6), 1063–1071.

Song, N., Wang, Z.M., He, L.J., Xu, Y., & Ren, Y.L. (2017). Estradiol enhanced osteogenesis of rat bone marrow stromal cells is associated with the JNK pathway. *Molecular Medicine Reports,* 16: 8589-8594.

Trivedi, R., Kumar, S., Kumar, A., Siddiqui, J.A., Swarnkar, G., & Gupta, V. (2008). Kaempferol has osteogenic effect in ovariectomized adult Sprague–Dawley rats. *Molecular and Cellular Endocrinology.* 289, 85–93.

Vijayan, V., Khandelwal, M., Manglani, K., Singh, R.R., Gupta, S., & Surolia A. (2013). Homocysteine alters the osteoprotegerin/RANKL system in the osteoblast to promote bone loss: pivotal role of the redox regulator forkhead. *Free Radical Biology and Medicine* 16, 72–84.

Yang, H.J., Kim, M.J., Qiu, J.Y., Zhang, T., Wu, X. & Jang, D. (2019). Rice porridge containing Welsh onion root water extract alleviates osteoarthritis-related pain behaviors, glucose levels, and bone metabolism in osteoarthritis-induced ovariectomized rats, *Nutrients,* 11(7), 1503.

Yang, Z., Zhang, Z., Penniston, K.L. Binkley, N. & Tanumihardjo, S A. (2008). Serum Carotenoid Concentrations in Postmenopausal Women from the United States with and without Osteoporosis. *Int. J. Vitam. Nutr. Res.,* 78 (3), 105–111.