

**Title of Study:** Mango will improve bone parameters in ovariectomized mice, a model of osteoporosis in postmenopausal women

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## **INTRODUCTION**

Osteoporosis is a medical condition due to loss of bone tissue causing the bone to become fragile and easy to fracture or break. According to The International Osteoporosis Foundation, osteoporosis causes more than 8.9 million fractures annually, resulting in osteoporotic fracture every 3 seconds.<sup>1</sup> The number of individuals affected by osteoporosis in Europe, USA, and Japan are estimated to be approximately 75 million.<sup>1</sup> In addition, the average cost of osteoporosis treatments and injury management can be extremely expensive depending on the type of treatment.<sup>1</sup>

Postmenopausal osteoporosis accounts for approximately 80% of osteoporosis cases that develop.<sup>1</sup> The risk for osteoporosis increases when women reach the age of menopause due to the decrease in production of the sex hormone estrogen. There are many physiological changes that affect bone due to estrogen deficiency. Changes in calcium balance, increase production of inflammatory factors and reactive oxygen species, increase activity of bone-breaking cells (osteoclasts) and decrease activity of bone-forming cells (osteoblasts) all occur due to estrogen deficiency and these changes compromise bone quality.<sup>2</sup>

There are several drugs available for treatment of osteoporosis. These drugs can either inhibit bone breakdown (anti-resorptive medications) or increase the rate of bone formation. For example, the drug alendronate is used to inhibit bone breakdown and increase bone mass which can reduce bone fracture. However, it has been reported that alendronate have adverse side effects such as joint or muscle pain and gastrointestinal complications (e.g. nausea, heartburn, abdominal pain, and ulcer).<sup>3</sup> Therefore, interest in seeking natural alternatives for the prevention or treatment of osteoporosis is gaining popularity.

One alternative approach for maintaining skeletal health that can delay or even prevent postmenopausal osteoporosis is the consumption of diet rich in fruits and vegetables.<sup>4-5</sup> Research studies have suggested the effectiveness of fruits such as blueberries and plums in improving bone parameters in postmenopausal women and rat model of postmenopausal osteoporosis.<sup>4-5</sup> Another fruit that may have a potential in sustaining skeletal health is the mango fruit. Mango (*Mangifera indica*) is high in antioxidants (vitamins A and C), minerals (calcium, phosphorus), as well as phenolic compounds (quercetin and mangiferin).<sup>6-7</sup> These antioxidants and other nutrients found in mango may help counteract the negative effects of free radicals and inflammatory molecules which can compromise skeletal health. We have previously shown that mango was able to modulate blood glucose similar to the glucose-lowering medication without compromising bone parameters in mice fed high fat diet.<sup>8</sup> The effects of mango and its polyphenol in preventing bone loss in ovariectomized (Ovx) mice, a model of postmenopausal osteoporosis bone parameters, were investigated in this study. We hypothesized that the mango fruit contains bioactive components that are capable of preventing postmenopausal osteoporosis.

## **APPROACH**

Ninety, 12-week old, C57/BL6 mice were obtained from Jackson Laboratories (Bar Harbor, ME) and acclimated for three days prior to removal of ovaries (ovariectomy) or sham surgery (underwent the same surgery but the ovaries were not removed). After surgery, mice were randomly assigned to one of the following treatment groups (n=12 mice/group): a) Sham-control, (b) Ovx-control, (c) Ovx+1% or 10% (w/w) freeze-dried mango or mango PP diet (equivalent PP to that of 1 and 10% mango), and (d) Ovx +alendronate (ALN) injection (0.1 mg/kg/week).

Mango (Tommy Atkins variety) was purchased from a local grocery store, peeled, and the pulp was freeze-dried, ground, analyzed for its nutrient composition and incorporated into the diet at 1% or 10% concentration by weight. Polyphenol was extracted from the mango pulp by homogenizing with acetone and methanol, extracted with hexane, and the aqueous phase was concentrated, filtered, and freeze-dried. Polyphenol was added to the control diet at doses equivalent to the amount found in 1% and 10% mango diets. All diets have the same macronutrient composition, as well as calcium and phosphorus. Ovariectomized mice were match-fed to the food intake of the sham group and reverse osmosis water were provided *ad libitum*. Mice were weighed weekly. After twelve weeks of treatment, mice were sacrificed and tissues were collected and various bone parameters were assessed.

## **RESULTS**

The findings of this study were presented at several local and national meetings.

1. Eldoumi H, Meister M, Peterson S, Ketz-Riley C, Perkins-Veazie, Clarke SL, Smith BJ, Lucas EA. Effects of mango on bone parameters of ovariectomized mice. Oklahoma State University Research Week, February 21, 2013, Stillwater, OK
2. Eldoumi H, Meister M, Peterson S, Ketz-Riley C, Perkins-Veazie, Clarke SL, Smith BJ, Lucas EA. Effects of mango on bone parameters of ovariectomized mice. Oklahoma Research Day, University of Central Oklahoma, March 12, 2013, Edmond, OK.
3. Eldoumi H, Meister M, Peterson S, Ketz-Riley C, Perkins-Veazie, Clarke SL, Smith BJ, Lucas EA. The effects of freeze-dried mango on bone parameters of ovariectomized mice. Oklahoma Skeletal Biology Symposium, November 4, 2013, Oklahoma City, OK.

4. Eldoumi H, Meister M, Peterson S, Ketz-Riley C, Perkins-Veazie P, Clarke SL, Smith BJ, Lucas EA. The effects of freeze-dried mango on bone parameters of ovariectomized mice. OSU Research Symposium, February 2014; Stillwater, OK.

5. Eldoumi H, Meister M, Peterson S, Ketz-Riley C, Perkins-Veazie P, Clarke SL, Smith BJ, Lucas EA. The effects of freeze-dried mango on bone parameters of ovariectomized mice. Experimental Biology, April 2014; San Diego, CA.

#### *Body Weight, Food Intake and Uterine Weight (Table 1)*

Mice had similar body weight at the start of treatment. The food intake of all the ovariectomized mice is similar and lower than the Sham group. Despite the lower food of the ovariectomized groups, they had higher body weight compared to the sham group after 12 weeks of dietary treatment. The significant difference in uterine weights ( $P < 0.0010$ ) between the Sham and Ovx groups indicate the success of the surgery.

#### *Tibial and Lumbar Mineral Content (BMC), Area (BMA), and Density (BMD) (Table 2)*

Menopause is associated with a slow and steady reduction in BMC and BMD due to the imbalance between bone formation and resorption rates. As expected, the Sham group had higher whole body BMC and BMA compared to the OVX groups. Freeze-dried mango or its polyphenol was not able to prevent the decrease in whole body BMC or BMA due to ovariectomy. BMD, which is calculated from BMC and BMA, was similar for all groups.

For all other bone analyses, the tibia (rich in cortical bone) and 4<sup>th</sup> lumbar vertebra (rich in trabecular or spongy bone) were chosen. Similar to the whole body, ovariectomy caused a significant decrease in proximal tibial and lumbar BMD. Mice fed 10% mango or 10% PP showed similar tibial BMD levels to ALN and Ovx-control groups. Among the Ovx groups,

lumbar BMD was highest in the ALN group followed by those mice fed 10% freeze-dried mango.

*Tibial and Lumbar Microarchitectural Parameters (Table 3 and Figure 1)*

In addition to changes in BMD, estrogen deficiency is also associated with compromised bone microarchitecture. Estrogen deficiency is characterized by a reduction in bone volume per total volume (BV/TV), connectivity density (Conn.Dens.), trabecular number (Tr.N.), trabecular thickness (Tr.Th) and increase in trabecular separation (Tr.Sp).<sup>9</sup> These changes result to a weaker bone. Micro-computed tomography analyses show that 10% mango and both doses of PP was able to improve lumbar BV/TV and Conn. Dens (similar to the alendronate treated group but still similar to the Ovx-control; Figure 1a and 1c). Tibial BV/TV and Conn. Dens were also improved by the PP treatments similar to the Sham group but also similar to the Ovx-control group.

As expected, lumbar Tr.N was decreased and Tr.Sp was increased due to ovariectomy (Figure 1b and Table 3). These parameters was brought to the levels of the Sham-control and ALN (but still similar to the Ovx-control mice) in mice that received the 10% mango and PP diets. Tibial Tr.Th, and Tb.Sp were similar among all the treatment groups.

The 10% mango diet was also able to improve the structural model index (SMI) of the lumbar bone similar to the ALN group. A low SMI indicates a plate-like trabeculae which provides more support and increases overall bone quality while a high SMI indicates a rod-like trabecular bone which decreases the support and bones become more vulnerable to fracture.<sup>10</sup> There were no differences in tibial SMI among all the treatment groups.

### *Bone Biomarkers and Anti-oxidant Enzymes (Table 4)*

Plasma procollagen type 1 amino-terminal propeptide (PINP), which is released from procollagen during collagen synthesis and is considered an indicator of bone formation, was measured. Additionally, pyridinoline (PYD), a bone resorption marker released during the breakdown of hydroxy lysyl crosslinks, was also assessed. The mango-fed groups have the same plasma PINP to that of the Sham group. There was no significant difference in the bone resorption marker PYD among all the treatment groups.

To determine if the effects of mango on bone is due to its potential influence on oxidative status, superoxide dismutase (SOD) and catalase activity were assessed on tibial extract. Mango and PP groups have statistically similar activity of the anti-oxidant enzyme SOD to the Sham group. However, no significant differences were observed in CAT activity among all the treatment groups.

### **Conclusion**

The findings of this study demonstrate that supplementation of freeze-dried mango in ovariectomized mice, a model of postmenopausal osteoporosis, has modest effects on bone. The higher dose of mango and its PP was able to slightly improve bone microarchitectural parameters which may help against bone fracture. Whether higher doses of mango are needed to have a more profound effect needs to be explored in future studies.

**Table 1** Effects of 12-week freeze-dried mango supplementation on body weight, food intake, and uterine weight of ovariectomized (ovx) mice<sup>1</sup>

Group/variable	Sham-control	Ovx-Control	Ovx-1%mango	Ovx-10%mango	Ovx-1%PP	Ovx-10%PP	Ovx-ALN	<i>P-value</i>
Initial body weight (g)	21.1 ± 0.6	21.6 ± 1.8	21.6 ± 1.2	22.0 ± 1.6	21.5 ± 1.2	21.6 ± 1.4	21.0 ± 0.8	0.7396
Final body weight (g)	25.3 ± 2.5 <sup>c</sup>	32.0 ± 4.4 <sup>ab</sup>	32.8 ± 3.2 <sup>a</sup>	32.2 ± 5.4 <sup>ab</sup>	34.8 ± 1.5 <sup>a</sup>	32.6 ± 1.7 <sup>a</sup>	29.2 ± 3.6 <sup>b</sup>	< 0.0001
Food intake (g)	3.5 ± 0.2 <sup>a</sup>	3.1 ± 0.2 <sup>b</sup>	3.2 ± 0.2 <sup>b</sup>	3.2 ± 0.3 <sup>b</sup>	3.2 ± 0.2 <sup>b</sup>	3.2 ± 0.2 <sup>b</sup>	3.3 ± 0.4 <sup>ab</sup>	0.0250
Uterine weight (mg)	121 ± 46 <sup>a</sup>	21 ± 12 <sup>b</sup>	16 ± 7 <sup>b</sup>	21 ± 14 <sup>b</sup>	15 ± 5 <sup>b</sup>	16 ± 5 <sup>b</sup>	12 ± 7 <sup>b</sup>	< 0.0001

<sup>1</sup>Values are mean ± SD; n= 9-11 mice/group. Within a row, values that do not have the same letters are statistically different (P<0.05).

PP - diet containing mango polyphenolic extract equivalent to the 1% or 10% mango diet, ALN - alendronate injection.

**Table 2** Effects of 12-week freeze-dried mango supplementation on whole body, proximal tibial and lumbar (L4) bone mineral content (BMC), area (BMA), and density (BMD) ovariectomized (ovx) mice <sup>1</sup>

Parameters	Sham-control	Ovx-control	Ovx-1%mango	Ovx-10%mango	Ovx-1%PP	Ovx-10%PP	Ovx-ALN	P-value
<b>Whole Body</b>								
BMC (mg)	685.7 ± 56.8 <sup>a</sup>	491.7 ± 58.5 <sup>bc</sup>	457.0 ± 66.0 <sup>c</sup>	478.1 ± 72.7 <sup>c</sup>	445.8 ± 59.0 <sup>c</sup>	473.0 ± 52.0 <sup>c</sup>	538.1 ± 45.6 <sup>bc</sup>	< 0.0001
BMA (cm <sup>2</sup> )	11.90 ± 0.66 <sup>a</sup>	8.91 ± 0.54 <sup>c</sup>	8.58 ± 0.34 <sup>c</sup>	8.74 ± 0.60 <sup>c</sup>	8.61 ± 0.27 <sup>c</sup>	8.74 ± 0.24 <sup>c</sup>	10.28 ± 0.68 <sup>b</sup>	< 0.0001
BMD (mg/cm <sup>2</sup> )	57.56 ± 1.94	55.11 ± 5.06	52.91 ± 6.97	54.49 ± 4.67	51.80 ± 6.70	54.08 ± 5.91	52.29 ± 1.76	0.220
<b>Tibia</b>								
BMC (mg)	21.7 ± 3.0	19.8 ± 2.0	19.8 ± 1.5	20.2 ± 1.9	20.3 ± 0.8	20.4 ± 1.1	21.4 ± 3.1	0.258
BMA (cm <sup>2</sup> )	0.445 ± 0.053	0.446 ± 0.044	0.445 ± 0.018	0.441 ± 0.019	0.455 ± 0.016	0.454 ± 0.15	0.455 ± 0.051	0.946
BMD (mg/cm <sup>2</sup> )	49.62 ± 1.40 <sup>a</sup>	44.83 ± 2.57 <sup>bc</sup>	44.55 ± 1.65 <sup>c</sup>	46.21 ± 3.87 <sup>bc</sup>	44.50 ± 1.91 <sup>c</sup>	45.06 ± 1.58 <sup>bc</sup>	46.88 ± 2.89 <sup>b</sup>	< 0.0001
<b>Lumbar</b>								
BMC (mg)	18.8 ± 2.1 <sup>a</sup>	14.8 ± 1.5 <sup>b</sup>	13.6 ± 1.0 <sup>b</sup>	15.25 ± 3.0 <sup>b</sup>	14.2 ± 1.8 <sup>b</sup>	14.1 ± 1.4 <sup>b</sup>	17.4 ± 2.8 <sup>a</sup>	< 0.0001
BMA (cm <sup>2</sup> )	0.319 ± 0.032	0.320 ± 0.025	0.316 ± 0.028	0.312 ± 0.039	0.313 ± 0.035	0.317 ± 0.025	0.319 ± 0.034	0.998
BMD(mg/cm <sup>2</sup> )	59.42 ± 3.38 <sup>a</sup>	46.25 ± 4.30 <sup>cd</sup>	43.10 ± 2.94 <sup>d</sup>	47.88 ± 4.35 <sup>c</sup>	45.65 ± 2.46 <sup>cd</sup>	44.36 ± 1.99 <sup>d</sup>	54.04 ± 4.96 <sup>b</sup>	< 0.0001

<sup>1</sup>Values are mean ± SD; n= 9-11 mice/group. Within a row, values that do not have the same letters are statistically different (P<0.05). PP - diet containing mango polyphenolic extract equivalent to the 1% or 10% mango diet, ALN - alendronate injection.

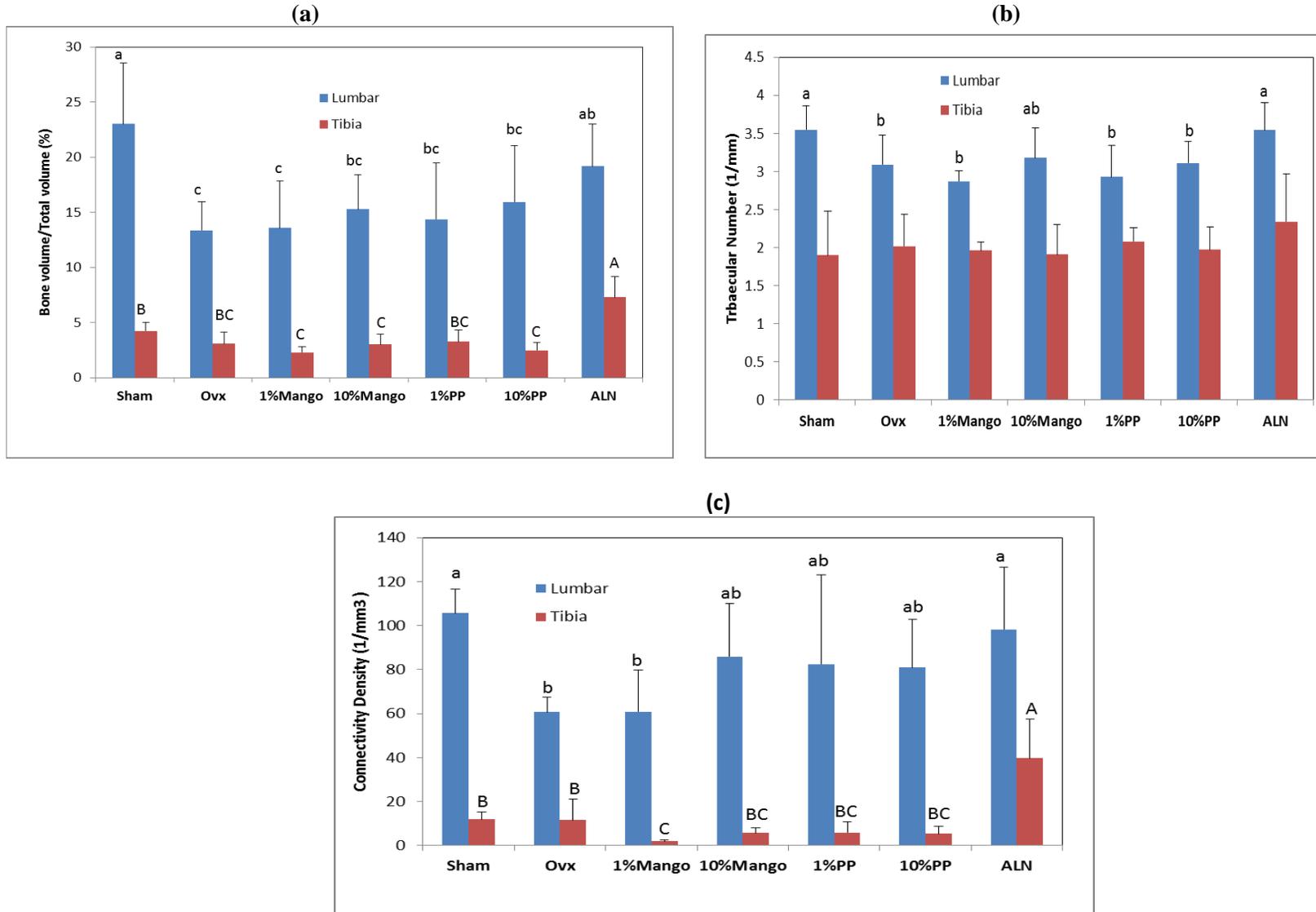
**Table 3** Effects of 12-week freeze-dried mango supplementation on tibial and lumbar (L4) microarchitectural parameters of ovariectomized (ovx) mice <sup>1</sup>

Parameters	Sham-control	Ovx-control	Ovx-1%mango	Ovx-10%mango	Ovx-1%PP	Ovx-10%PP	Ovx-ALN	<i>P-value</i>
<b>Lumbar</b>								
SMI	0.90 ± 0.42 <sup>c</sup>	1.75 ± 0.25 <sup>a</sup>	1.82 ± 0.42 <sup>a</sup>	1.50 ± 0.42 <sup>ab</sup>	1.58 ± 0.45 <sup>ab</sup>	1.49 ± 0.40 <sup>ab</sup>	1.19 ± 0.49 <sup>bc</sup>	0.008
Tb.Sp. ( <i>um</i> )	289.0 ± 30.1 <sup>b</sup>	334.7 ± 42.2 <sup>a</sup>	355.4 ± 17.0 <sup>a</sup>	327.4 ± 41.7 <sup>ab</sup>	352.2 ± 56.9 <sup>a</sup>	328.9 ± 26.3 <sup>ab</sup>	286.3 ± 31.8 <sup>b</sup>	0.015
TrTh ( <i>um</i> )	63.0 ± 8.3	55.8 ± 5.8	55.8 ± 7.4	54.5 ± 5.0	53.5 ± 3.4	56.0 ± 6.6	57.3 ± 2.0	0.155
<b>Tibia</b>								
SMI	2.31 ± 0.39	2.51 ± 0.37	2.70 ± 0.14	2.50 ± 0.24	2.47 ± 0.24	2.60 ± 0.22	2.40 ± 0.55	0.5515
Tb.Sp. ( <i>mm</i> )	0.62 ± 0.23	0.52 ± 0.14	0.51 ± 0.02	0.52 ± 0.08	0.48 ± 0.03	0.52 ± 0.06	0.40 ± 0.11	0.138
TrTh ( <i>mm</i> )	48.7 ± 5.1	46.9 ± 3.5	54.0 ± 6.4	52.6 ± 6.2	50.6 ± 2.0	50.1 ± 3.2	47.4 ± 7.6	0.223

<sup>1</sup>Values are mean ± SD; n= 6 mice/group. Within a row, values that do not have the same letters are statistically different (P<0.05).

PP - diet containing mango polyphenolic extract equivalent to the 1% or 10% mango diet; ALN - alendronate injection; SMI – structure model index; TbSp- trabecular separation; TbTh- trabecular thickness.

**Figure 1** Effects of 12-week freeze-dried mango supplementation on tibial and lumbar (L4) (a) bone volume/total volume, (b) trabecular number and (c) connectivity density of ovariectomized (ovx) mice <sup>1</sup>



<sup>1</sup>Values are mean  $\pm$  SD; n= 6 mice/group. Bars that do not have the same letters are statistically different (P<0.05). Small and capital letters are comparison for lumbar and tibial bone, respectively.

PP - diet containing mango polyphenolic extract equivalent to the 1% or 10% mango diet; ALN - alendronate injection

**Table 4** Effects of 12-week freeze-dried mango supplementation on bone biomarkers and anti-oxidant enzymes of ovariectomized (ovx) mice <sup>1</sup>

Parameters	Sham-control	Ovx-control	Ovx-1%mango	Ovx-10%mango	Ovx-1%PP	Ovx-10%PP	Ovx-ALN	<i>P-value</i>
<b>Plasma</b>								
PYD (nmol/L)	4.93 ± 0.88	4.53 ± 0.78	3.92 ± 0.97	5.75 ± 1.80	3.96 ± 1.47	4.15 ± 1.27	5.29 ± 2.29	0.057
PINP (ng/mL)	24.25 ± 5.64 <sup>a</sup>	22.69 ± 4.91 <sup>a</sup>	23.21 ± 6.07 <sup>a</sup>	20.63 ± 4.11 <sup>ab</sup>	17.49 ± 4.61 <sup>b</sup>	14.83 ± 3.41 <sup>bc</sup>	12.53 ± 2.97 <sup>c</sup>	< 0.0001
<b>Tibial Extract</b>								
CAT ( nmol/ min/mL )	7.15 ± 2.47	5.86 ± 2.19	5.68 ± 0.71	6.42 ± 2.53	7.65 ± 4.11	7.48 ± 3.58	8.30 ± 2.09	0.694
SOD (U/mL)	0.31 ± 0.08 <sup>abc</sup>	0.36 ± 0.08 <sup>a</sup>	0.31 ± 0.04 <sup>abc</sup>	0.27 ± 0.08 <sup>bc</sup>	0.32 ± 0.07 <sup>ab</sup>	0.26 ± 0.06 <sup>c</sup>	0.32 ± 0.04 <sup>ab</sup>	0.018

<sup>1</sup>Values are mean ± SD; n= 9-11 mice/group. Within a row, values that do not have the same letters are statistically different (P<0.05).

PP - diet containing mango polyphenolic extract equivalent to the 1% or 10% mango diet, ALN - alendronate injection; PINP- procollagen type 1 amino-terminal propeptide; PYD- pyridinoline; CAT- catalase; SOD- superoxide dismutase.

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