CHAPTER IV

RESULTS

Effect of AR root extract on femoral parameters

After exposing to the various dosages of AR root extract daily for 90 days, the right femur from all rats were dissected out and cleaned of soft tissues and fat for the femoral measurement of length, thickness and weight. The longitudinal length and the thickness at metaphysis of femurs were measured by using vernier caliper. The weight of femur was weighed using a weighing machine. Effects of AR root extract on ovariectomy-induced alterations in femoral length, thickness and weight of all the treatment groups are presented in Table 6. Regarding to femoral length, ovariectomy resulted in a reduction in the femoral length after 90 days of ovariectomy (3.65 ± 0.07 cm and 3.58 ± 0.05 cm for SHAM and OVX control rats, respectively). There was significant difference ($p < 0.05$) in the femoral length between OVX control and OVX+AR100 group. Therefore, AR at 100 mg/kg B.W./day may prevent shortening of bone induced by ovariectomy. No significant change in femoral length was shown in OVX+AR1000 rats compared to that both SHAM and OVX control rats. Similarly, no significant change in femoral length was observed in OVX rats treated with EE at 0.1 mg/kg B.W./day compared to that both SHAM and OVX control rats.

In the measurement of femoral thickness, the femoral thickness of OVX control rats was similar to that of SHAM rats (0.58 ± 0.02 cm and 0.52 ± 0.02 cm for SHAM and OVX control rats, respectively). The femoral thickness of treated group with AR root extract at the dose level of 100 and 1000 mg/kg B.W./day were 0.62 ± 0.03 cm and 0.60 ± 0.01 cm, respectively. Compared to OVX control rats, treatment with AR root extract at 100 mg/kg B.W./day prevented the femoral thickness reduction and this difference was statistically significant ($p < 0.01$). Likewise, OVX rats treated with AR (1000 mg/kg B.W./day) prevented the femoral thickness reduction and this difference was statistically significant ($p < 0.05$) when compared to OVX control. AR treatment in all dosing groups significantly improved the femoral thickness. The thickness of femurs in OVX rats treated with EE was 0.56 ± 0.02 cm.

Femoral thickness showed a tendency to increase in the OVX+EE rats compared to OVX control rats but with no significant difference.

Considering femoral weight, the greatest reduce in femoral weight occurred in OVX control rats as the mean value for this group was significantly different ($p < 0.01$) from the mean value for rats in SHAM group (0.93 ± 0.02 g and 0.81 ± 0.04 g for SHAM and OVX control rats, respectively). The femoral weight of treated group with AR root extract at the dose level of 100 and 1000 mg/kg B.W./day were 0.91 ± 0.03 g and 0.95 ± 0.01 g, respectively. Compared to SHAM rats, significantly increase ($p < 0.05$) of femoral weight was observed in OVX rats treated with 100 mg/kg B.W./day of AR root extract. Likewise, there was significant difference ($p < 0.01$) between OVX control and OVX+AR1000 group, showing an enhancing of femoral weight on treated animals. Significant dose-dependent in femoral weight was shown in AR-treated groups (OVX+AR100 group and OVX+AR1000 group). There was between OVX control and OVX+EE group there was significant difference ($p < 0.01$), demonstrating an elevation of femoral weight of ovariectomized rat after EE administration.

Table 6 Effect of AR root extract on femoral parameters after 90 days of treatment

Treatment groups	Length (cm)	Thickness (cm)	Weight (g)
SHAM	3.65 ± 0.07	0.58 ± 0.02	0.93 ± 0.02
OVX	3.58 ± 0.05	0.52 ± 0.01	0.81 ± 0.04^{b}
OVX+AR100	$3.77 \pm 0.04^{\#}$	$0.62 \pm 0.03^{\#\#}$	$0.91 \pm 0.03^{a,\#}$
OVX+AR1000	3.67 ± 0.05	$0.60 \pm 0.01^{\#}$	$0.95 \pm 0.01^{a,\#\#}$
OVX+EE	3.65 ± 0.06	0.56 ± 0.02	$0.95 \pm 0.01^{a,\#\#}$

Each value is express as mean ± S.E.M. of six animals. $^{a}p < 0.05$ and $^{b}p < 0.01$ compared to SHAM; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to OVX.

Effect of AR root extract on biochemical parameters

β-CTx level

Biochemical markers of bone turnover respond sensitively toward change in bone remodeling. There are many types of markers that most commonly used for bone resorption. Serum β-CTx, the established marker for bone resorption was determined in this study. The results of serum β-CTx concentration in animals of various groups are shown in Figure 17. Regarding the serum β-CTx analysis, the OVX control rats showed the highest concentration of β-CTx level and was significantly different ($p < 0.001$) from rats in the SHAM group (0.23 ± 0.04 ng/ml and 0.46 ± 0.01 ng/ml for SHAM and OVX control rats, respectively). The concentration of serum β-CTx in OVX+AR100 and OVX+AR1000 group were 0.34 ± 0.01 ng/ml and 0.35 ± 0.02 ng/ml, respectively. Animals receiving with either 100 or 1000 mg/kg B.W./day of AR root extract showed significant elevation ($p < 0.05$) in the concentration of serum β-CTx compared to that of SHAM rats. The treatment with AR (100 and 1000 mg/kg B.W./day) showed reduction in serum β-CTx level with statistical significance ($p < 0.01$) in comparison with those in OVX control rats. Furthermore, the concentration of serum β-CTx in OVX rats treated with EE was lower than that of OVX control rats. The difference was statistically significant ($p < 0.001$).

P_1NP level

P_1NP is secreted by osteoblasts and is markers of bone formation. Serum P_1NP has emerged as a reliable marker of bone turnover in humans and animals and is routinely used to monitor bone formation. The results of serum P_1NP concentration of various groups of rats are shown in Figure 18. The results demonstrated an elevation in the concentration of serum P_1NP level in OVX control rats as compared to that of SHAM rats (13.78 ± 3.49 ng/ml and 30.75 ± 4.42 ng/ml for SHAM and OVX control rats, respectively). The level of serum P_1NP between SHAM and OVX control rats differed significant ($p < 0.01$). The concentration of serum P_1NP of treated group (with AR root extract at the dose level of 100 and 1000 mg/kg B.W./day) were 27.47 ± 4.03 ng/ml and 26.21 ± 1.94 ng/ml, respectively. Compared to SHAM rats, significantly increases ($p < 0.01$) of serum P_1NP level was observed in OVX+AR100 group. Similarly, compared to SHAM rats, significantly difference ($p < 0.05$) was found in the serum P_1NP level in OVX+AR1000 group. The concentration

of serum P_1NP tended to be lower in AR-treated groups (100 and 1000 mg/kg B.W./day) than that of OVX control rats but this trend did not achieve statistical significance. Feeding of EE to OVX rats significantly reduced ($p < 0.01$) the concentration of serum P_1NP compared to OVX control rats.

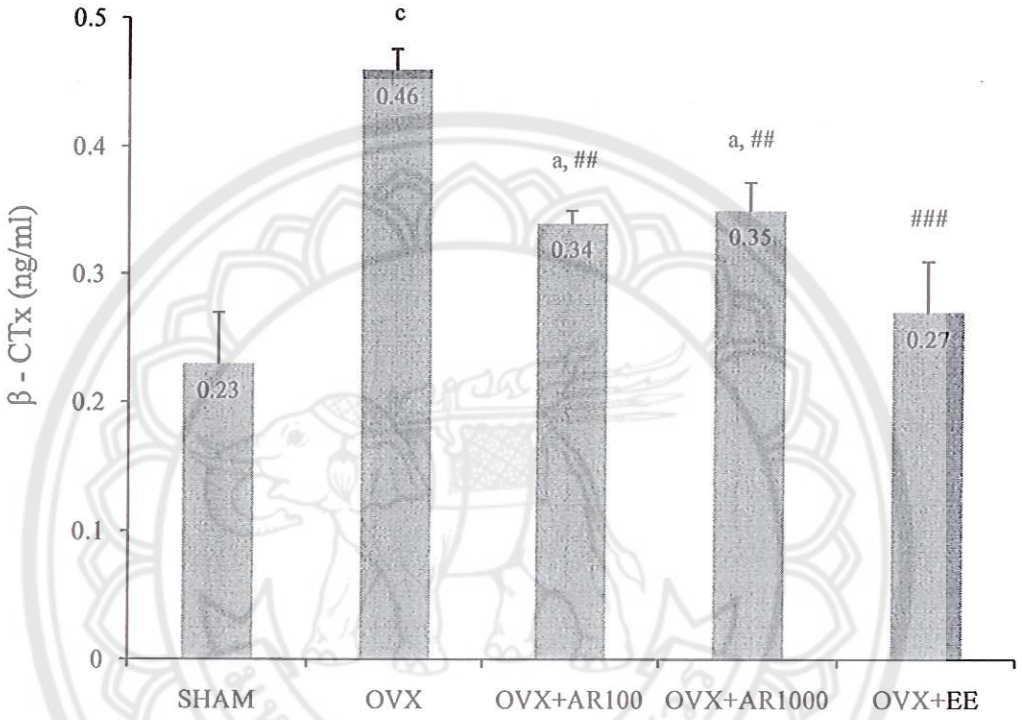

Figure 17 Effect of AR root extract treatment on serum β-CTx measured in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals. $^{a}p < 0.05$ and $^{c}p < 0.001$ compared to SHAM; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared to OVX.

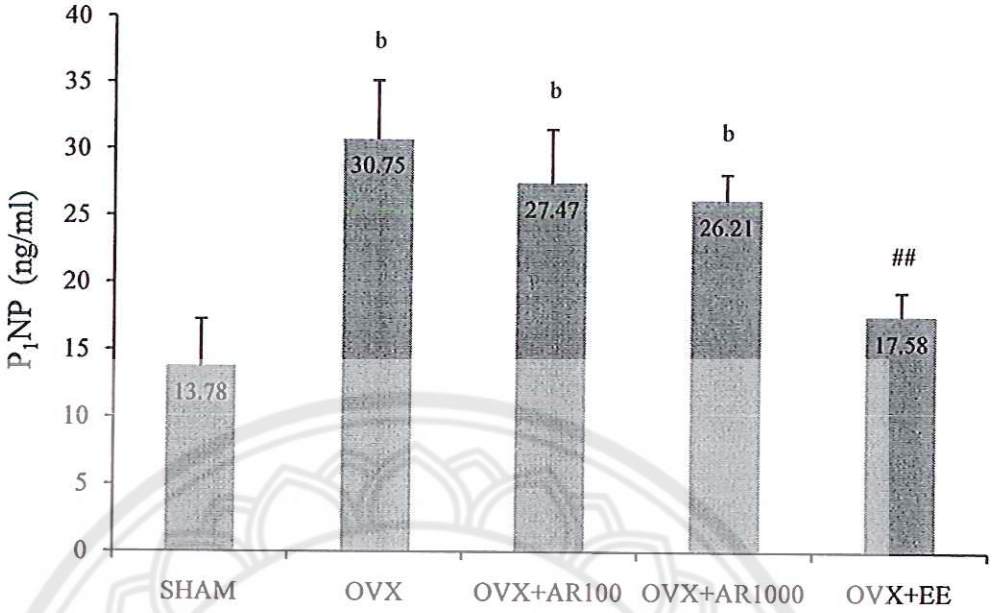

Figure 18 Effect of AR root extract treatment on serum P_1NP measured in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals. $^{b}p < 0.01$ compared to SHAM; $^{\#\#}p < 0.01$ compared to OVX.

Effect of AR root extract on Calcium, phosphorus, ALP and estradiol level

Effects of AR root extract on calcium, phosphorus, ALP and estradiol level are summarized in Table 7. Analyzing the level of serum calcium, the difference between SHAM and OVX control rats had no statistical significance (10.06 ± 0.45 and 10.31 ± 0.19 mg/dL for SHAM and OVX control rats, respectively). There was no significant change in the serum concentration of calcium in all treatment groups except OVX rats treated with EE

In the serum phosphorus measurement, the difference between SHAM and OVX control rats had no statistical significance (4.00 ± 0.34 mg/dL and 4.51 ± 0.31 mg/dL for SHAM and OVX control rats, respectively). Treatment with EE to OVX rats did not show any significant change in serum phosphorus. Serum phosphorus level was significantly increased ($p < 0.05$) in OVX+AR100 group compared to that of

OVX control rats as well as in OVX+AR1000 group (5.56 ± 0.30 mg/ml and 5.56 ± 0.18 mg/ml, for OVX+AR100 and OVX+AR1000 group, respectively).

Regarding to serum ALP measurement, the serum ALP concentration was similar between SHAM and OVX control rats (53.83 ± 4.19 and 58.50 ± 5.28 for SHAM and OVX control rats, respectively). In the case of the treatment groups, the concentration of serum ALP of treated group (with AR root extract at the dose level of 100 and 1000 mg/kg B.W./day) were 45.66 ± 4.66 U/L and 45.16 ± 5.66 U/L, respectively. Although not significant, a slight decrease in serum ALP was observed in AR-treated rats when compared to OVX control rats. Administration of EE significantly reduced ($p < 0.01$) the concentration of serum ALP compared to that of SHAM rats. The OVX+EE group also showed the least concentration of serum ALP level and was significantly different ($p < 0.01$) from OVX control group.

Analyzing the concentration of serum estradiol, OVX control rats showed significant reduction ($p < 0.05$) in estradiol level compared to that of SHAM rats (22.91 ± 5.63 pg/ml and 13.13 ± 1.95 pg/ml for SHAM and OVX control rats, respectively). Treatment of AR root extract either 100 or 1000 mg/kg B.W./day also decreased the concentration of serum estradiol from those in SHAM group. Moreover, the level of serum estradiol in both OVX+AR100 and OVX+AR1000 groups was similar to that of OVX control group.

Table 7 Effects of AR root extract on calcium, phosphorus, ALP and estradiol after 90 days of treatment

Treatment groups	Calcium (mg/dL)	Phosphorus (mg/dL)	ALP (U/L)	Estradiol (pg/ml)
SHAM	10.06 ± 0.45	4.00 ± 0.34	53.83 ± 4.19	22.91 ± 5.63
OVX	10.31 ± 0.19	4.51 ± 0.31	58.50 ± 5.28	13.13 ± 1.95[a]
OVX+AR100	10.30 ± 0.20	5.56 ± 0.30[a]	45.66 ± 4.66	12.71 ± 1.97[a]
OVX+AR1000	10.08 ± 0.20	5.56 ± 0.18[a]	45.16 ± 5.66	13.77 ± 1.51[a]
OVX+EE	8.06 ± 1.62[#]	4.31 ± 0.88	32.33±6.94[b,##]	16.09 ± 2.55

Each value is expressed as mean ± S.E.M. of six animals. [a]$p < 0.05$ compared to SHAM; [#]$p < 0.05$ and [##]$p < 0.01$ compared to OVX.

Histological analysis of femur

The histological morphology of distal femur in all experimental groups was illustrated in Figure 19 to Figure 23. The femoral longitudinal-section revealed normal size, shape, density and architecture of trabecular bone with intertrabecular spaces in SHAM rats (Figure 19). In addition, the spaces between the trabecular bones, the marrow cavity of the epiphysis, is filled by bone marrow or adipose tissues. Highly dense marrow spaces were seen in SHAM group. The femoral-longitudinal section of OVX control rats (Figure 20) exhibited the thinner and smaller trabecular bone which is resulted in wider intertrabecular spaces and loss of marrow spaces formation after 90 days of ovariectomy. As in the case of the treatment group (Figure 21 and 22), the thick elongated trabecular bone with narrowed intertrabecular spaced were found in the OVX rats treated with either 100 or 1000 mg/kg B.W./day of AR root extract when compared to that of OVX contol rats. Animals that were received with EE (Figure 23) revealed the thicker trabeculae with high connectivity and narrowed intertrabecular spaces compared with those of OVX control rats. Moreover, improved the thickness of trabecular bone with almost dense marrow spaces were observed in OVX rats treated with EE.

Figure 19 Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was SHAM operated control and orally administered with vehicle for 90 days.

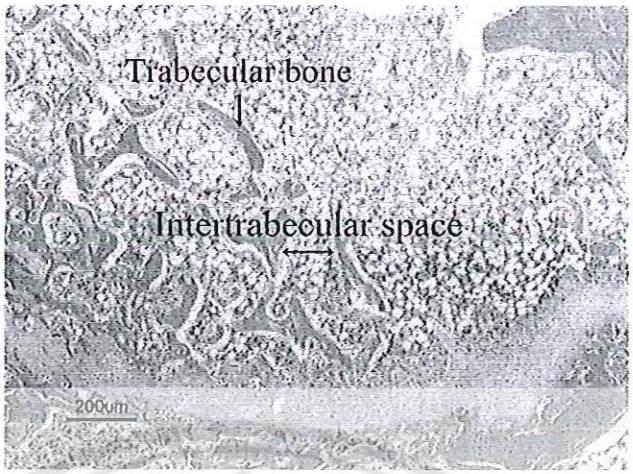

Figure 20 Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy.

Figure 21 Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.

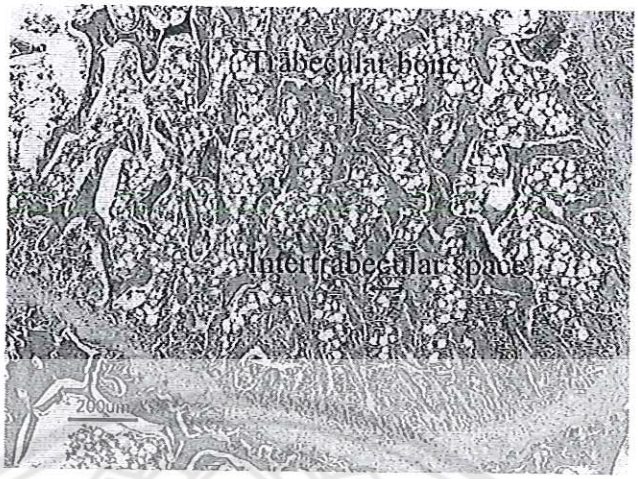

Figure 22 Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.

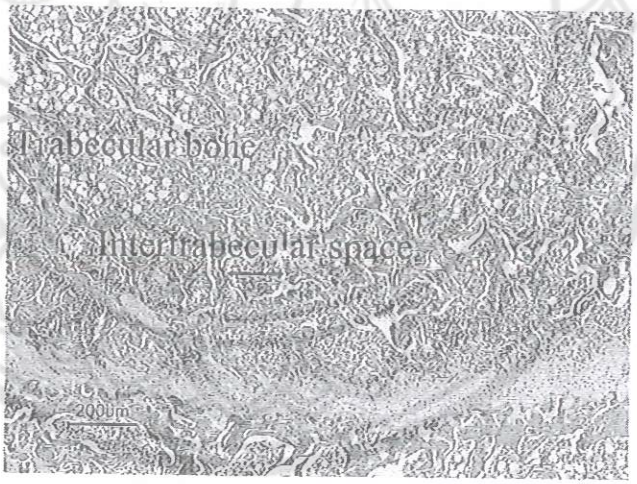

Figure 23 Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy.

Histomorphometric analysis of trabecular bone

This morphological observation was quantitated by histomorphometric analysis of longitudinal-sections obtained from the distal femur. The distal femur was assessed for trabecular thickness, trabecular space and trabecular area. The results of quantitative trabecular bone histomorphometric measurement are shown in Figure 24 to Figure 26.

Trabecular thickness

Effects of AR root extract on trabecular thickness in different groups of rats are shown in Figure 24. Ovariectomy caused a dramatically decreased in the thickness of trabecular bone compared to that of SHAM rats (135.76 ± 7.04 μm and 58.00 ± 4.28 μm for SHAM and OVX control rats, respectively). There was significant difference (p <0.001) between SHAM and OVX control rats. In the case of the treatment groups, trabecular thickness of rats fed with AR root extract at the dose level of 100 and 1000 mg/kg B.W./day were 94.61 ± 6.24 μm and 101.41 ± 4.32 μm, respectively. Significantly increased (p < 0.001) of trabecular thickness was found in OVX+AR100 rats in comparison with those in OVX control rats. Treatment with AR root extract at 1000 mg/kg B.W./day also increased (p < 0.001) the thickness of trabecular bone compared with those in OVX control group. These results showed that trabecular thickness in all dosing groups of AR were thicker than those of OVX control rats. EE administration absolutely prevented the decreased in trabecular thickness. The thickness of trabecular bone in this group was thicker than OVX control rats and this showed statistical significance (p < 0.001).

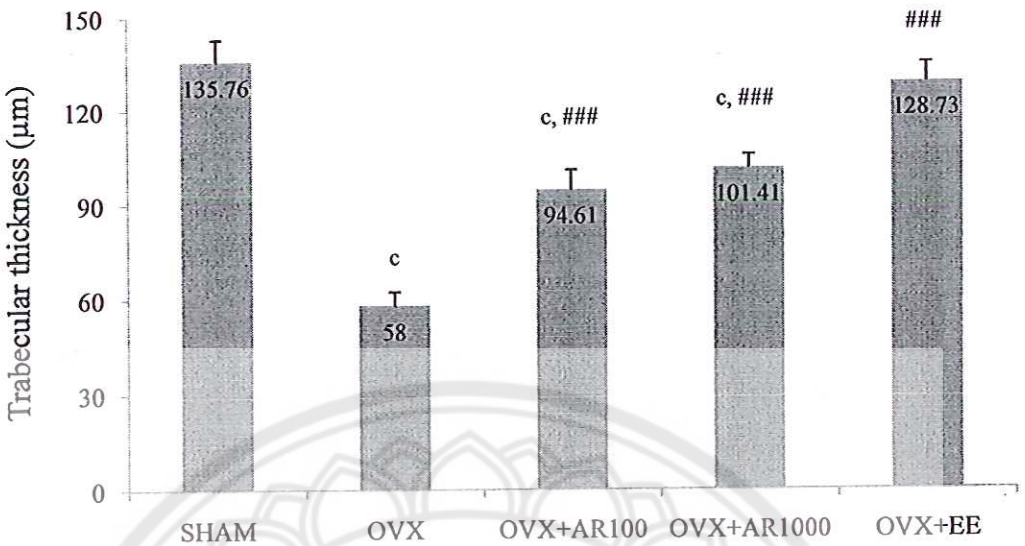

Figure 24 Effect of AR root extract on the thickness of trabecular bone in various groups of rats. Treatments represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals. $^{c}p < 0.001$ compared to SHAM; $^{###}p < 0.001$ compared to OVX.

Intertrabecular space

Effects of AR root extract on trabecular space in different groups of rats are shown in Figure 25. After 90 days of ovariectomy, intertrabecular space in OVX control rats was dramatically increased in comparison with those in SHAM group (84.20 ± 5.51 µm and 153.90 ± 14.57 µm for SHAM and OVX control rats, respectively). Intertrabecular space of SHAM and OVX control rats differed significantly ($p < 0.001$). Intertrabecular space was decreased in OVX+AR100 rats when compared to that of OVX control rats but the difference was not statistically significant. Compared to OVX control rats, administration of AR root extract at 1000 mg/kg B.W./day to OVX rats caused decreased in intertrabecular space. The intertrabecular space between OVX control and OVX+AR1000 rats differed significantly ($p < 0.05$). These results showed that the rats in treated groups had less intertrabecular space than OVX control rats. Moreover, intertrabecular space in feeding of EE was narrower than those of OVX control rats. This difference was statistically significant ($p < 0.01$).

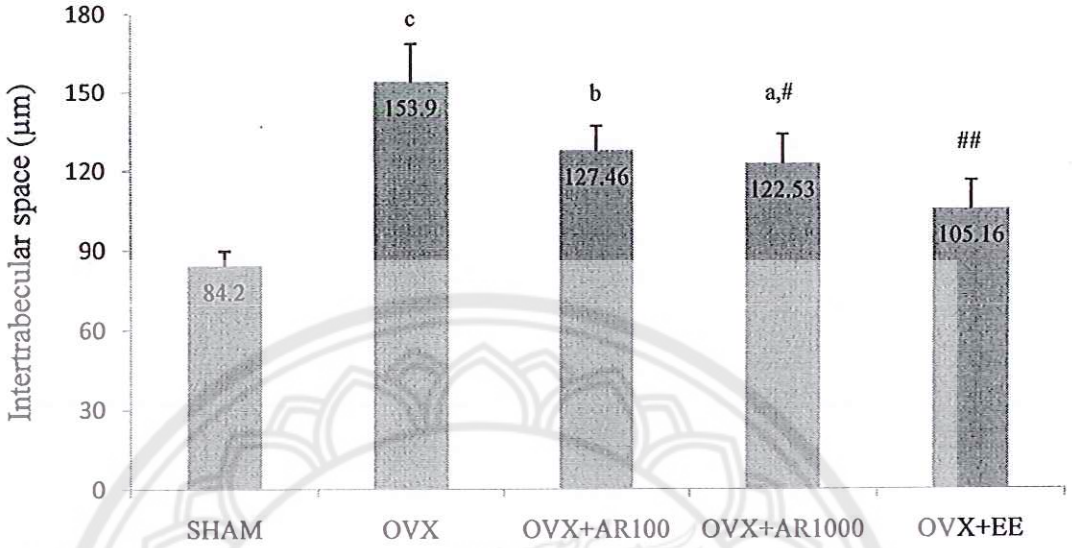

Figure 25 Effect of AR root extract on intertrabecular space in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ compared to SHAM; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to OVX.

Trabecular area

The trabecular areas in metaphyseal region of distal femurs in rats following various treatments for 90 days are presented in Figure 26. Areas were expressed as a percentage of the total area that was examined. The greatest decrease in trabecular area found in OVX control rats as the mean value for this group was significantly different (p <0.001) from the mean value for SHAM rats (57.25 ± 5.03 mm^2 and 16.82 ± 0.67 mm^2 for SHAM and OVX control rats, respectively). Trabecular area in AR-treated OVX rats (100 mg/kg B.W./day) was significantly increased ($p < 0.01$) compared to that of OVX control rats. Similarly, trabecular area was significantly increased (p <0.001) in AR-treated OVX rats (1000 mg/kg B.W./day) relative to OVX control rats after 90 days of treatment (30.32 ± 1.20 μm^2 and 36.01 ± 1.67 μm^2 for OVX+AR100 and OVX+AR1000 group, respectively). Treatment of OVX rats with EE had increased (p <0.001) the trabecular area by over the rats in OVX control group.

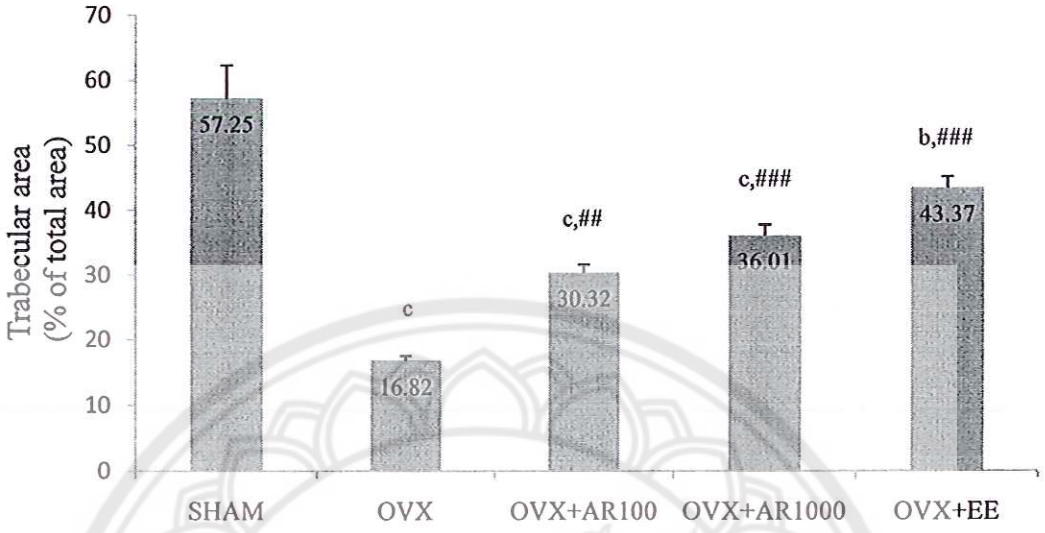

Figure 26 Effect of AR root extract on trabecular area in metaphysis of distal femur in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals. $^{b}p < 0.01$ and $^{c}p < 0.001$ compared to SHAM; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared to OVX.

Histological analysis of cortical bone

To evaluate the protective effects of AR root extract on bone loss, this study examined the effect of AR root extract on the microstructural change not only in cancellous (trabecular) bone but also in compact (cortical) bone of rat after ovariectomy.

The section of cortical bone in longitudinal plane of femur was stained with H&E and examined under a light microscope. Figure 27 to Figure 31 illustrated the histological study of the cortical bone in SHAM and OVX rats were gavaged daily for 90 days with vehicle, AR root extract at 100 and 1000 mg/kg B.W./day and EE at 0.1 mg/kg B.W./day, respectively. Histological section of SHAM rats (Figure 27) revealed normal compactness of cortical bone and trabecular bone with high connectivity under the influence of endogenous estrogens. The matrix decalcified bone is strongly eosinophilic because of its high content of collagen. No difference in the cortical bone

histological characteristics can be observed between SHAM and OVX control rats (Figure 28). Similarly, there were no difference in the cortical bone histological characteristics in AR-treated groups (100 and 1000 mg/kg B.W./day) (Figure 29 and 30) when compared to SHAM and OVX control rats. In animals treated with EE (Figure 31), the histology of cortical bone was similar to that of SHAM and OVX control group.

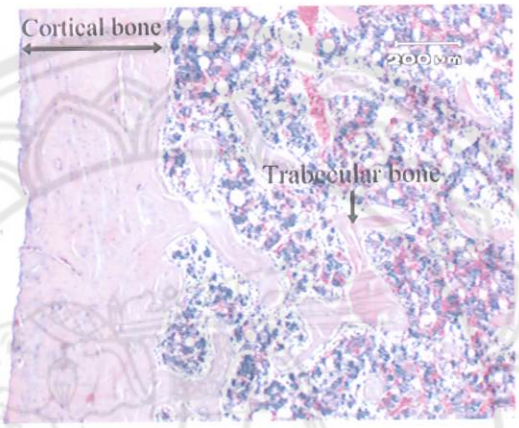

Figure 27 Cortical bone morphology in longitudinal plane of femur in animal that was SHAM operated control and orally administered with vehicle for 90 days.

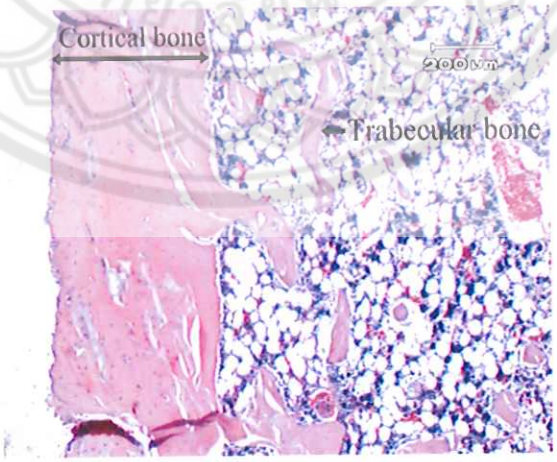

Figure 28 Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy.

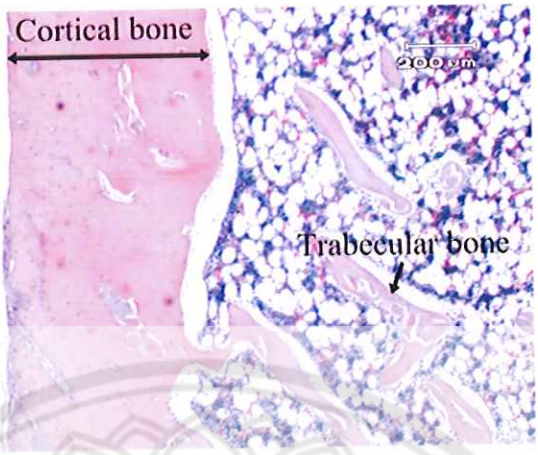

Figure 29 Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.

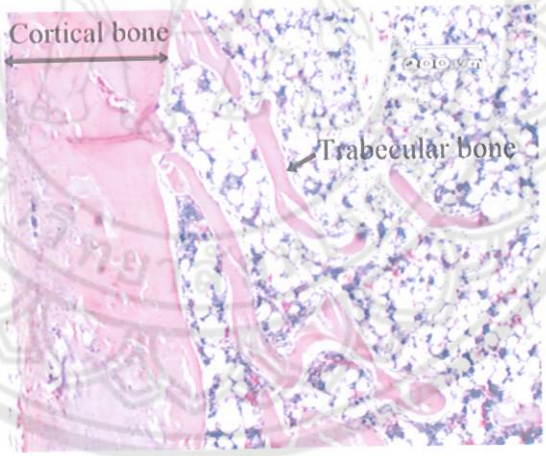

Figure 30 Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.

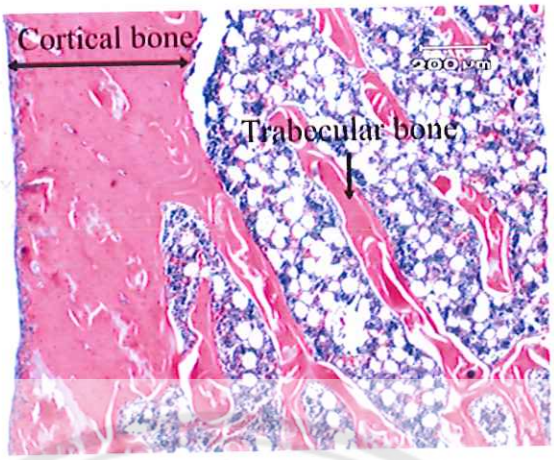

Figure 31 Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy.

Histomorphometric analysis of cortical bone thickness

In the cortical bone, the histological characteristics showed no change in rats of all groups. As a result, to evaluate whether the treatment with AR root extract at two different dose levels of 100 and 1000 mg/kg B.W./day had any effect on cortical bone. The histomorphometry of cortical bone thickness was observed in this study.

The results of cortical bone thickness are shown in Figure 32. In the measurement of cortical bone, the thickness was not significantly different in SHAM rats relative to OVX control rats (299.84 ± 12.87 μm and 309.96 ± 15.30 μm for SHAM snd OVX control rats, respectively). Also, no significant alteration was observed in cortical bone thickness in OVX rats treated with AR root extract either at 100 or 1000 mg/kg B.W./day. The thickness of cortical bone was thickest in the treatment with EE but the difference was not statistically significant.

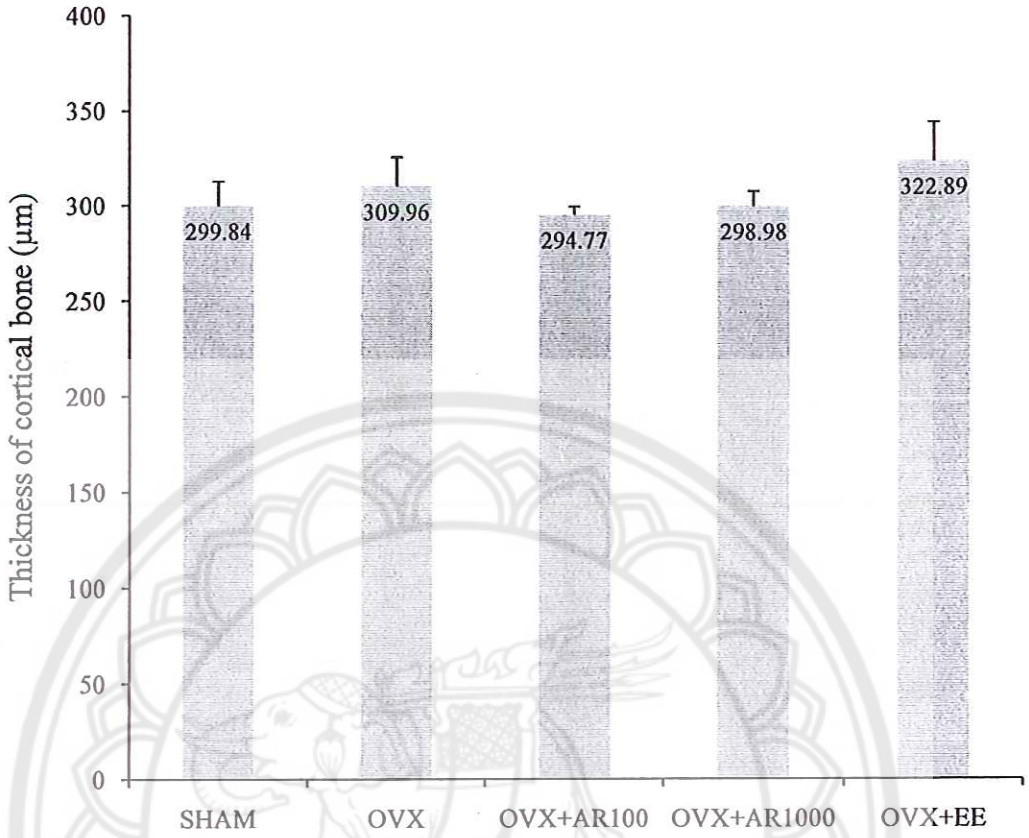

Fig 32 Effect of AR root extract on cortical bone thickness in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals.

Body weight

At the beginning of the experiment the adult female Wistar rats were 90 days of age with an average weight between 250 g to 300 g. Body weight measurements for all the group of animals were weighed once a week throughout the treatment period and finally at the end of treatment. The body weights of different groups of rats are presented in Table 8. In the SHAM and OVX control rats, the final body weights were 304.97 ± 5.67 g and 333.15 ± 8.34 g, respectively. Bilateral OVX induced statistically significant ($p < 0.05$) increase in final body weight in female rats. Gain in body weight also showed in OVX control rats after 90 days of ovariectomy as comparable to SHAM rats (42.15 ± 5.28 g and 60.80 ± 7.28 g for SHAM and OVX control rats,

respectively). Animals treated with all doses of AR showed gain in body weight but this alteration was less than that in OVX control rats. In EE treated group, the body weight was 270.61 ± 2.17 g. Treatment with EE cause greatly decreased in body weight and this difference was statistically significant ($p < 0.001$).

Table 8 Effects AR root extract on rats body weight after 90 days of treatment

Treatment groups	Initial B.W. (g)	Final B.W. (g)	Weight gain (g)
SHAM	262.82 ± 6.49	304.97 ± 5.67	42.15 ± 5.28
OVX	272.34 ± 4.43	333.15 ± 8.34[a]	60.80 ± 7.28
OVX+AR100	277.28 ± 9.07	326.93 ± 14.14	49.64 ± 17.28
OVX+AR1000	282.81 ± 7.10[a]	334.30 ± 10.53[a]	51.49 ± 14.53
OVX+EE	286.42 ± 4.63[a]	270.61 ± 2.17[b,###]	-15.80 ± 4.90[b,###]

Note: Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Each value is expressed as mean ± S.E.M. of six animals. [a]$p <$ 0.05 and [b]$p < 0.01$ compared to SHAM; [###]$p < 0.001$ compared to OVX.

Uterine weight

At the end of treatment, the uterus was dissected out and immediately weighed. The changes in uterine weight of different groups of rats are shown in the Table 9. Ninety days after ovariectomy, the uterine weights of SHAM and OVX control rats were 813.03 ± 157.35 mg and 338.38 ± 188.59 mg, repectively. Uterine weight of OVX control rats was greatly decreased in comparison with the SHAM rats and this difference was statistically significant ($p < 0.01$). The uterine weights in group treated with AR root extract at 100 and 1000 mg/kg B.W./day were 127.03 ± 27.37 mg and 173.05 ± 7.80 mg, respectively. The weights of the uterus in all doses of AR root extract were similar to that of OVX control rats. Treatment with AR did not alter the uterine weight. Between SHAM and OVX+EE group, there was no significant difference in uterine weight.

Table 9 Effects AR root extract on rats uterine weight after 90 days of treatment

Treatment groups	Absolute uterine weight (mg)	Relative uterine weight (mg/100 g B.W.)
SHAM	813.03 ± 157.35	2.67 ± 0.53
OVX	338.38 ± 188.59[b]	1.02 ± 0.57[b]
OVX+AR100	127.03 ± 27.37[c]	0.38 ± 0.09[c]
OVX+AR1000	173.05 ± 7.80[c]	0.52 ± 0.03[c]
OVX+EE	554.53 ± 37.06	2.03 ± 0.14[##]

Note: Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Each value is expressed as mean ± S.E.M. of six animals. [b]$p <$ 0.01 and [c]$p < 0.001$ compared to SHAM; [##]$p < 0.01$ compared to OVX.

As shown in Table 9, the results of absolute uterine weight similar to the relative uterine weight (mg/100 g B.W.). The OVX control rats showed the decreases of relative uterine weight and were significantly different ($p < 0.01$) from SHAM rats (2.67 ± 0.53 mg/100 g B.W. and 1.02 ± 0.57 mg/100 g B.W. for SHAM and OVX control rats, respectively). In the case of the treatment group, the relative weights of the uterus of oral administration of 100 and 1000 mg/kg B.W. of AR root extract were 0.39 ± 0.09 and 0.52 ± 0.03 mg/100 g B.W./day, respectively. Significantly decrease ($p < 0.001$) of relative uterine weight was demonstrated in all dosing group of AR compared to that of SHAM group. Furthermore, the relative uterine weight in both OVX+AR100 and OVX+AR1000 group was similar to that of OVX control group.

The difference in the absolute uterine weight of OVX rats treated with EE had no statistical significance as compared to both SHAM and OVX control rats. The relative uterine weight of animal treated with EE was 2.05 ± 0.14 mg/100 g B.W. Compared to OVX control rats, treatment with EE to OVX rats resulted in significant increase ($p < 0.01$) in relative weight of the uterus. These results showed that ovariectomy caused atrophy of the uterus and this was prevented by feeding of 0.1 mg/kg B.W./day of EE for 90 days to maintain uterine weight at the level of SHAM

rats. However, the present data showed that uterine weight in OVX rats treated with AR at both 100 and 1000 mg/kg B.W./day was slightly less than that of OVX control rats, yet dramatically less than that of SHAM rats.

Histological analysis of uterus

Measurement of the uterine weight showed that the administration in rats following various treatment of AR root extract had no uterotrophic effect. However, the undesirable adverse effects on the reproductive tissues after the use of AR root extract to prevent bone loss in OVX rats should be concerned if long-term feeding and various dosages are used. Hence, this study is necessary to be conducted to examine the effect of AR root extract on microstructural change in reproductive tissues of rats after ovariectomy.

The uterus is a major hormone-responsive reproductive sex organ. Myometrium and endometrium layers are sensitive to the fluctuating levels of estrogen secreted by ovary. Therefore, the morphology of endometrium and myometrium were observed for histological analysis in this study. Effect of AR root extract on uterine histology after 90 days of treatment was illustrated in Figure 33 to Figure 37. Under light microscope, the uterus of SHAM rats (Figure 33) had an outer thick muscular layer and contained a rich network of arteries and veins supported by dense collagenous tissue, the myometrium. Inner to myometrium, which is composed of inner mucosal layer and contained uterine glands, the endometrium. The luminal surface of the endometrium is lined by a simple columnar epithelium. Outermost, serosal layer, or perimetrium, that is formed by peritoneum. The peritoneum extends laterally and form broad ligament of the uterus. It attaches the uterus to the sides of the pelvis and contains nerve as well as blood and lymphatic vessels passing to the uterus. As seen in micrograph (Figure 34), the section of uterus in OVX control rats had a smaller size of uterus than those of SHAM rats. Moreover, the sparse uterine glands were found in this group. Uterine size in OVX+AR100 group (Figure 35) was much smaller than those of SHAM group as well as in OVX+AR1000 group (Figure 36). Uterine size of all dosing group of AR root extract was similar to OVX control rats. Furthermore, decrease in endometrial glands in OVX+AR100 and OVX+AR1000 groups were similar to OVX control rats. The results showed that the uterus of AR

root extract at either 100 or 1000 mg/kg B.W./day-treated animals had a similar morphologic pattern to that of OVX control rats. In contrast, treatment with EE to OVX rats (Figure 37) maintained uterine size at the level of SHAM rats. Increase in endometrial glands was found in OVX+EE group when compared to OVX control group.

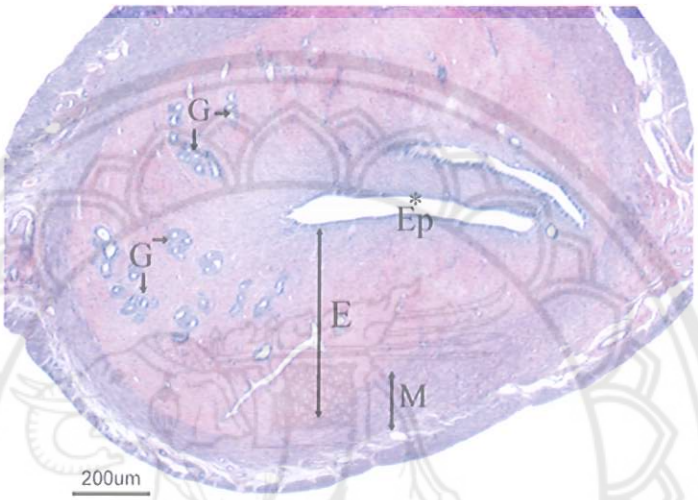

Figure 33 Uterine morphology in transverse plane in animal that was SHAM operated control and orally administered with vehicle for 90 days. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).

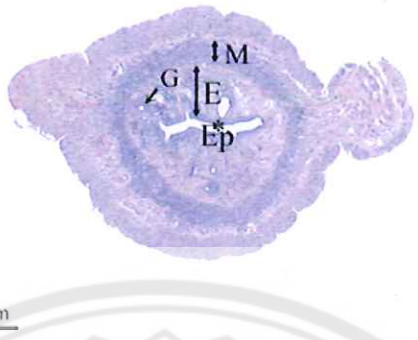

Figure 34 Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).

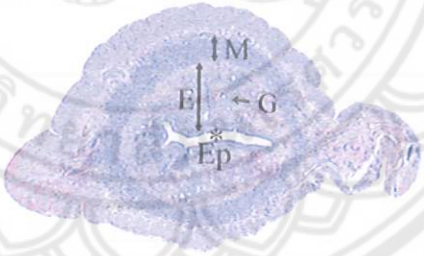

Figure 35 Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).

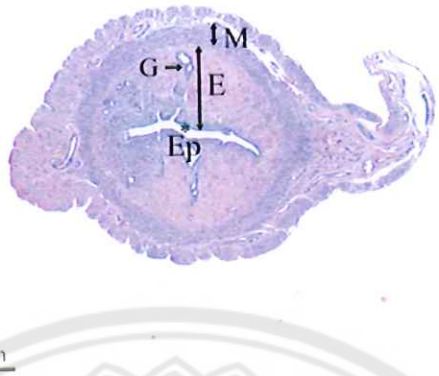

Figure 36 Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).

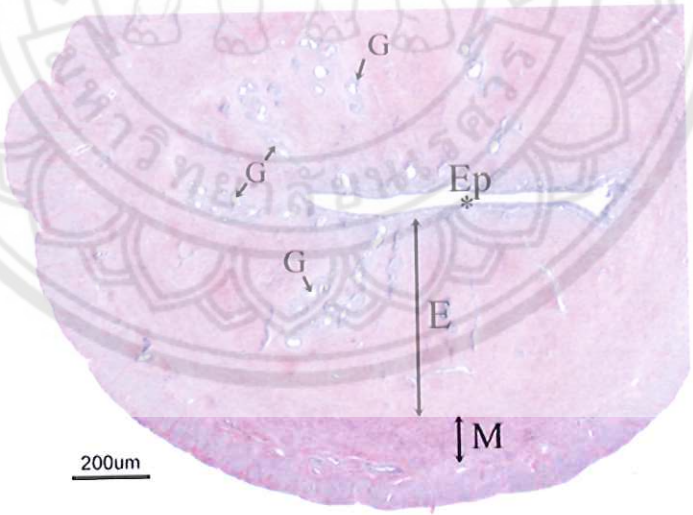

Figure 37 Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).

Histomorphometric analysis of uterus

The thickness of endometrium and myometrium of uterine wall was quantitated in this study to confirm that the feeding of AR root extract at various doses ranging from 100 and 1000 mg/kg B.W./day for 90 days had no proliferative effect on uterus.

The thickness of the endometrium and myometrium is summarized in Table 10. Quantitatively, endometrium thickness dramatically decreased ($p < 0.001$) in OVX control rats when compared to SHAM rats (560.10 ± 6.43 μm and 187.90 ± 5.48 μm for SHAM and OVX control rats, respectively). The thickness of myometrium in OVX control rats also significantly ($p < 0.001$) reduced in comparison with those in SHAM rats (102.37 ± 5.93 μm and 60.93 ± 3.47 μm for SHAM and OVX control rats, respectively). Compared to SHAM rats, OVX+AR100 group showed greatly decrease ($p < 0.001$) in their thickness of endometrium, similar to that of OVX+AR1000 group. Uterine cross-sectional endometrium and myometrium thickness in feeding of AR root extract at the dose level of 100 and 1000 mg/kg B.W./day were able to maintain the uterine layers close to that of OVX control rats. Besides, administration of EE was able to maintain the thickness of endometrium and myometrium of uterine wall at the levels of SHAM rats.

Table 10 Effects of AR root extract on thickness of endometrium and myometrium after 90 days of treatment

Treatment groups	Endometrium thickness	Myometrium thickness
SHAM	560.10 ± 6.43	102.37 ± 5.93
OVX	187.90 ± 5.48^{c}	60.93 ± 3.47^{c}
OVX+AR100	212.00 ± 2.82^{c}	57.18 ± 3.01^{c}
OVX+AR1000	219.48 ± 2.30^{c}	65.12 ± 3.96^{c}
OVX+EE	544.50 ± 21.78$^{###}$	99.87 ± 5.28$^{###}$

Note: Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Each value is expressed as mean ± S.E.M. of six animals. $^{c}p < 0.001$ compared to SHAM; $^{###}p < 0.001$ compared to OVX.

Histological analysis of mammary gland

Mammary gland is a target tissue that estrogen can influence. Ducts and lobuli of mammary gland develop mainly in rising levels of estrogen cause further branching. Secretion or increase in number of ducts was observed for confirming the stimulation. Therefore, to evaluated whether the feeding of AR root extract at the doses of 100 and 1000 mg/kg B.W./day for 90 days had any proliferative effect on mammary gland. The microstructural changes of mammary gland were observed in this study.

Figure 38 to Figure 42 showed histology of mammary gland in SHAM and OVX rats that gavaged daily for 90 days with vehicle, AR root extract (100 and 1000 mg/kg B.W./day) and EE (0.1 mg/kg B.W./day), respectively. Under the influence of endogenous estrogen in female rats (Figure 38), the mammary gland is mainly composed of ducts with very few acini. The ducts consist of one or two layers of epithelial cell which are cuboidal epithelial cells bordering the lumen. The duct is embedded in fibrocollagenous tissue. There is a surrounding zone of fibrocollagenous support tisssue, outside which is the soft adipose tissue. At this low magnification, greatest decrease in glandular area was seen in OVX control rats (Figure 39) in comparison with the SHAM group. The section of mammary gland in OVX control rats also showed no secretion in ducts. Animals fed with AR root extract both at 100 and 1000 mg/kg B.W./day (Figure 40 and 41) showed no secretion in ducts similar to OVX control rats. Furthermore, reduction in glandular area was found in AR treatment. All doses of AR root extract had a similar mammary gland morphological pattern to OVX control rats. Whereas, animals that were fed with EE at 0.1 mg/kg B.W./day (Figure 42) had an increase in proportions and enlarged ducts of mammary gland compared to that of SHAM and OVX control rats.

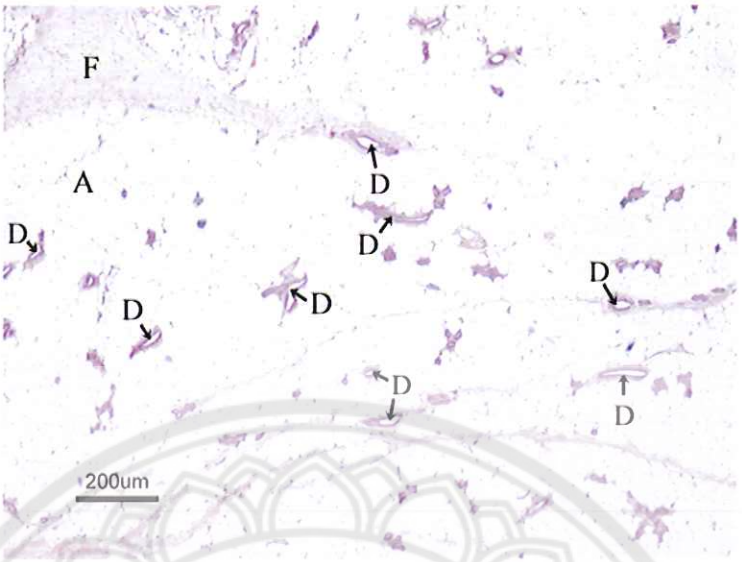

Figure 38 Mammary gland morphology in animal that was SHAM operated control and orally administered with vehicle for 90 days showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).

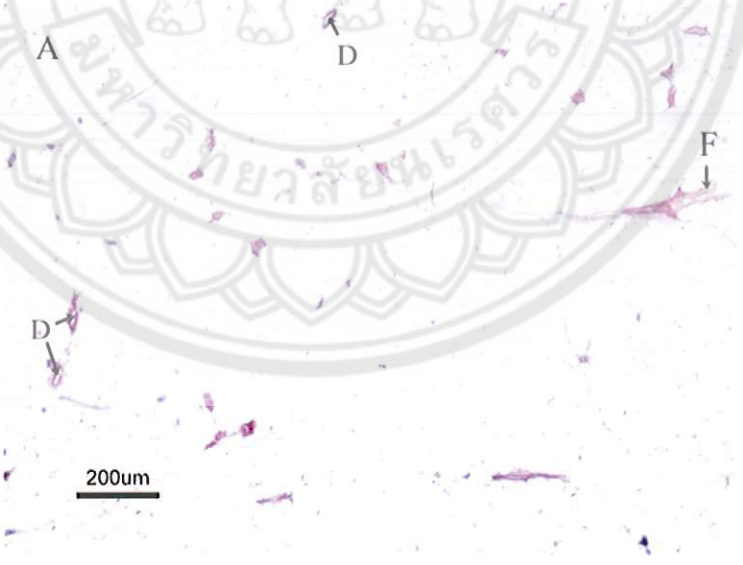

Figure 39 Mammary gland morphology in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).

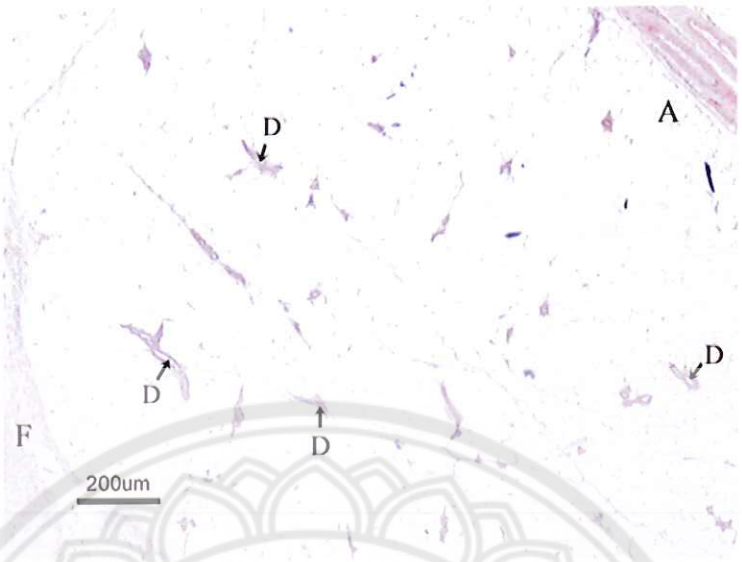

Figure 40 Mammary gland morphology in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).

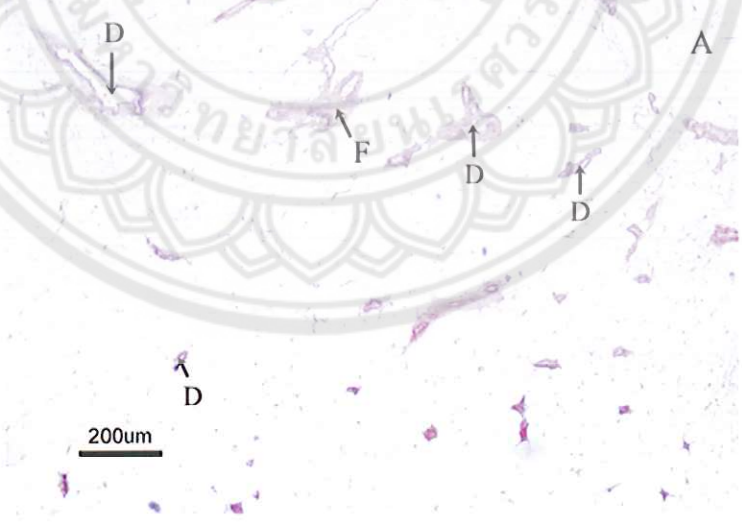

Figure 41 Mammary gland morphology in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).

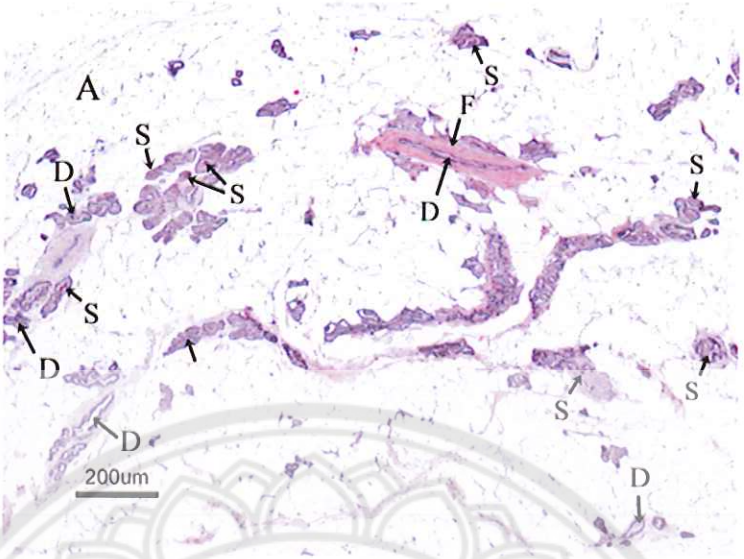

Figure 42 Mammary gland morphology in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A). Note the minute amount of secretion (S) in the lumen of duct resulting from stimulation of estrogen treatment.

Histomorphometric analysis of mammary gland

Figure 43 showed the glandular area measurements of mammary gland in all experimental groups. The ovariectomy resulted in a reduction in the glandular area compared to SHAM rats (5.67 ± 1.67 and 1.33 ± 0.88 for SHAM and OVX control rats, respectively). In OVX+AR100 and OVX+AR1000 groups, the glandular area was similar to OVX control rats. These results showed that AR root extract at two different dose levels did not produce any changes in this histomorphometric parameter. In OVX+EE group, there was a statistically significant increase ($p < 0.001$) in the glandular area by over the SHAM and OVX control rats. The glandular area was greatest in EE treatment.

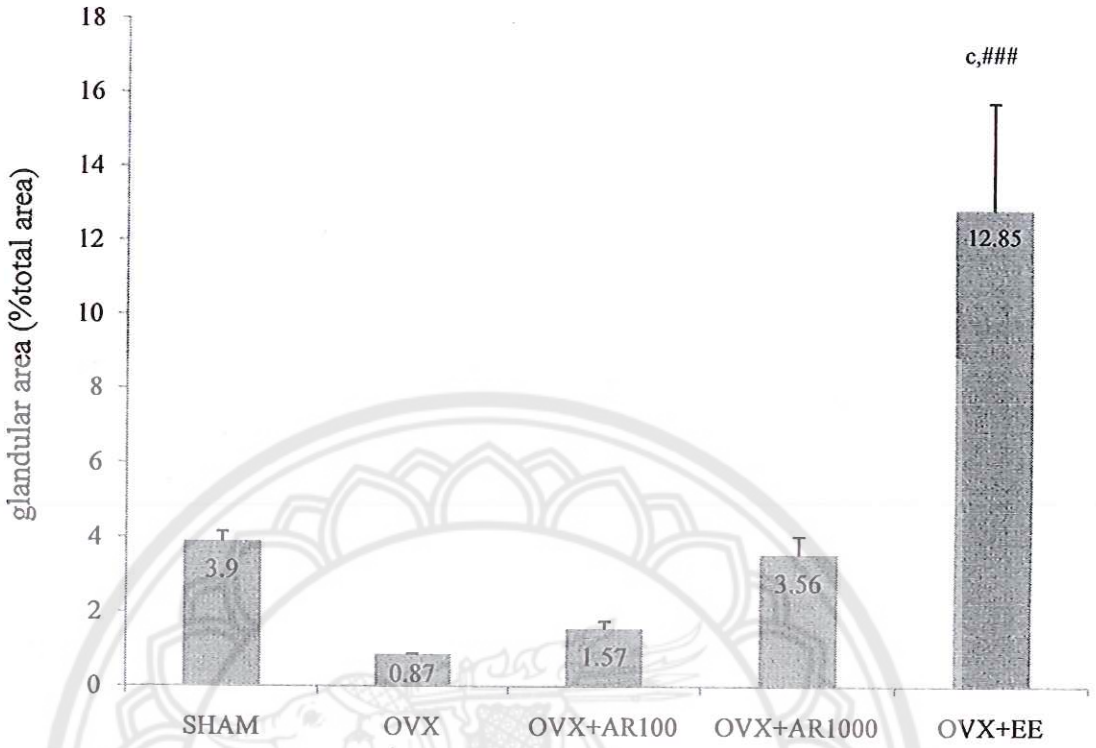

Figure 43 Effect of AR root extract on the glandular area of mammary gland. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean ± S.E.M. of six animals. $^{c}p < 0.001$ compared to SHAM; $^{###}p < 0.001$ compared to OVX.