CHAPTER V

DISCUSSIONS

Effect of AR root extract on femoral parameters

In this study, we have shown that the length, thickness and weight of femur were decreased. The decreased in these femoral parameters after ovariectomy was recovered by the treatment with AR. These results were comparable to the previous study by Chitme, Muchandi and Burli (2009) which demonstrated that OVX rats consistently showed higher femoral parameters when received with AR root extract. Moreover, our result showed that AR root extract was able to increase femoral weight in a dose-dependent manner. Normally, the change of bone weight was not a significant indicator to detect the efficacy of anti-osteoporotic agents except ash bone weight (Yamamoto, et al., 1998). However, significant increase trends of bone weights were found in all dosing groups of AR root extract in this study. Hence, it was considered that the increase trends of bone weight could be related to the preventive effect of AR root extract on bone loss.

Based on the results obtained, AR treatment could prevent both the decrease in femoral length and thickness in the OVX rats. The mechanisms regulating alteration in femoral parameters are unclear at present. AR root extract may stimulate the synthesis of growth hormone (GH), which is known to increase the longitudinal growth. Our finding was consistent with a study by Reddy, Lakshmana and Udupa (2004) that has revealed phytosterols contained in herbal formulation OST-6 (Osteocare) is effective in improving femoral length in OVX rats. They suggested that increased in the length of femur may be due to the stimulatory effect of OST-6 on GH.

Phytochemical substances have shown the presence of saponins, sterols, alkaloids, flavonoides and terpene (Bopana and Saxena, 2007). Based on the enzyme-linked immunosorbent assay (ELISA) technique analyzed by Onlom, et al. (2011), the phytoestrogens found in AR root extrac‘ in this study is total saponin. The percentage of total saponin in AR root extract was 7.42. Previous study demonstrated that major active component, Shatavarin IV analyzed in AR root by high performance liquid

chromatography (HPLC) as shown by the percentage of 0.17 (Hayes, et al., 2008). Furthermore, Chiba, et al. (2003) have shown that the natural steroids have been able inhibit bone loss. We provide the evidence that the preventive effect of AR root extract on bone loss should depend on quantities and types of phytoestrogens.

Effect of AR root extract on bone markers

Based on the results, following ovariectomy at the end of ninety days, a significant increase was found in serum β-CTx and P_1NP in comparison to SHAM group. These results are consistent with the previous studies in OVX rats, which reported that ovariectomy may have influence on both bone resorption and bone formation by increase in biochemical markers of bone turnover (Arjamandi, et al., 1995; Devareddy, et al., 2008; Kapur, et al., 2008). This is correspondence with a hypothesize reporting that as a consequence of ovarian hormone deficiency, the rate of bone resorption increases and then induce bone formation to happen. Therefore, elevated levels of both β-CTx and P_1NP, which can be measured in serum as markers is associated the change in bone remodeling.

In contrast with Arjamandi, et al. (1995), Karlsson, et al. (1999) and Kapur, et al. (2008), demonstrated that 17α-Ethynylestradiol (EE) prevent ovariectomy-induced elevation of biochemical markers. This study is also in confirmation with previous evidence by Arjamandi, et al. (1995), Devareddy, et al. (2008) and Kapur, et al. (2008), which showed that the treatment with EE significantly decreased the activity of both serum β-CTx and P_1NP in OVX rats relative to SHAM rats. The results of this study provided the evidence that estrogen treatment totally suppressed the rising of β-CTx and P_1NP levels induced by ovariectomy. Estrogen treatment maintains bone integrity and stabilizes the serum levels of the bone turnover.

According to the study, serum concentrations of both β-CTx and P_1NP were less in the AR-treated groups than that in OVX control group. It could be suggested that AR root extract was beneficial in normalizing the increased bone marker levels of both β-CTx and P_1NP. Nevertheless, AR root extract at both 100 and 1000 mg/kg B.W./day was not as efficient inhibit bone turnover as EE at 0.1 mg/kg B.W./day. Moreover, the concentration of serum β-CTx was decreased significantly in OVX rats treated with AR as compared to OVX control rats and the concentration of serum

P_1NP in OVX rats treated with AR was similar to that of OVX control rats. These results might indicate the preventive effect of AR root extract which may be due to suppress bone resorption with no effect in bone formation.

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

There were no significant differences in the serum calcium among any groups except OVX+EE group. Also, serum phosphorus level was not significantly affected by any of the treatments. These observations are supported by previous studies of Annie, Prabhu and Malini (2006), Zhang, et al. (2006) and Chitme, Muchandi and Burli (2009). They suggested that the unchanged levels of serum calcium and phosphorus indicated that homeostatic mechanisms were able to maintain serum levels of these minerals despite ovariectomy.

ALP levels have been shown to correlate with bone formation and resorption. Ninety days after ovariectomy, OVX rats showed an increase in ALP level relative to SHAM rats. On the other hand, between OVX control and AR-treated rats, showed a depleted for the concentration of serum ALP. Moreover, a significant decrease in ALP activity was presented in EE-treated animals. The results could be explained that EE treatment could reverse the increase of ALP activity induced by ovariectomy and AR treatment might have similar effects on ALP activity as EE. The result corresponded with the previous observations of Devareddy, et al. (2008), Kapur, et al. (2008), Nian, et al. (2009), Hassen and Saed (2011). The findings of this study indicated that elevated serum ALP levels are due to ovarian hormone deficiency and are prevented by estrogen administration. Moreover, our findings was consistent with a previous study that has revealed the treatment of OVX rats with methanolic and aqueous extract of AR show significant alteration in serum ALP level (Chitme, Muchandi and Burli, 2009). In addition, the increase in level of ALP was observed with respect to increased serum β-CTx and P_1NP in OVX control group. However, ALP is not specific marker to bone. There are several isoenzyme of ALP which comes from other tissue sources, particularly liver, kidney and intestine.

Estrogen deficiency due to bilateral ovariectomy was shown to cause significantly decreased in serum estradiol level in rat models. Serum estradiol level in EE-treated group was similar to that in SHAM group. It is apparent from the results

presented in this study that supplementation with EE usually maintained the serum estradiol level in OVX rats. In AR-treated rats, the serum estradiol was not significant different from OVX control rats. We have demonstrated that synthetic estrogen cause higher serum estradiol level than natural estrogen. No alteration in serum estradiol concentration being reported in this study are similar to that of reported by El-shitany, Hegazy, and El-desoky (2010) demonstrated that Silymarin, a mixture of four isomeric flavonolignans from milk thistle (*Silybum marinun*) did not change the reduced serum EE level in OVX rats.

Effect of AR root extract on femoral histology and histomorphometry

As seen in this study, the histological examination in vehicle-treated OVX rats showed evidence of disruption and deterioration in the architecture of trabecular bone along with loss of connectivity. All these changes showed that following ovariectomy, there is a considerable enhanced in bone fragility, implying that estrogen deficiency causes the development of osteoporosis in animal model. Moreover, the histological examination correlated with the biochemical study as characterized by an elevation in β-CTx, P_1NP and ALP concentration in OVX control rats. There are an increase in bone resorption and decrease in bone formation in ovariectomized rats (Annie, Prabhu and Malini, 2006).

Histology of the femur of AR-treated groups also revealed the restoration of trabecular network with thick elongated trabeculae and less intertrabecular spaces. These observations are evidence for marked restorative of bone loss and thereby suggested that the protective effect of AR may be due to an increase in bone formation and a reduction in bone resorption. In addition, femoral histology in the group treated with AR was observed with respect to decrease in biochemical markers of bone turnover thus showing an increase of osteoblastic activity and decrease of osteoclastic activity.

Ovariectomy decreased the histomorphometrical properties, such as the thickness, space and area of trabecular bone (Yin, et al., 2006; Zhao, et al., 2011) and the same was observed in this study. Bahuman and Wronski (1995) reported that the decrease of trabecular thickness and area and the increase of trabecular space in OVX control rats were induced by high bone turnover. As a positive control treatment in this

study, 0.1 mg/kg B.W./day of EE significantly increased trabecular thickness and area, and decreased trabecular space. Our finding was consistent with a previous study that has revealed EE at a dose level of 0.1 mg/kg B.W./day could prevent bone loss shown as the decreased of BMD and BMC of cortical and trabecular bone were prevented in aged OVX rats (Urasopon, et al., 2008).

OVX+AR100 and OVX+AR1000 groups kept better architecture of femur as comparable to OVX control group. These could be suggested that a lessening of thickness and area of trabecular bone and the widening of intertrabecular space in OVX rats was attenuated by AR root extract. It was reasonable to propose that AR root extract could act similarly to EE to prevent bone loss but EE is more potent in protecting the animal from bone loss than AR root extract.

Additionally, the effect of AR root extract on histology and histomorphometry of cortical bone was investigated. The findings of this study showed no significant alteration in these parameters in all groups. These imply that OVX resulted in alteration of trabecular bone more than cortical bone. Our observation was in agreement with the study of Urasopon et al. (2008). They found that the effects of ovariectomy on BMD and BMC are smaller in cortical compartments than in trabecular compartments. Interestingly, previous study on the earliest changes in the cortical bone width and the marrow cavity of the femoral and tibial shaft are noticed between 90 and 120 days after ovariectomy (Danielsen, Mosekilde and Svenstrup, 1993). Furthermore, Jee and Yao (2001) reported that cortical bone requires to 180 days or longer after surgery to achieve steady state.

Effect of AR root extract on body weight

Ninety days after ovariectomy, animals in the OVX control group had significantly greater final body weights than SHAM group. It is well documented that post ovariectomy, estrogen withdrawal leads to the accumulation of energy stores (fat deposition) and subsequently increase in the body weight of animals (Devareddy, et al., 2006). However, the exact mechanism by which OVX induces increase in body weight is not clear (Joyner, Hutley, and Cameron, 2001). The well-known elevation of body weight gain was a shift in energy metabolism due to ovarian hormone deficiency (Arjmandi, et al., 1997).

On the other hand, EE was found to maintain body weight close to SHAM rats. Our results agreed with the previous reports showing that the ovariectomy in rats can induce body weight gain and treatment of estrogen prevents excess weight gain (Arjmandi, et al., 1996). Estrogen was found to maintain the original body weight. It is likely that estrogens act by exerting anti-lipogenic effects on adipose tissue (Anwar, et al., 2001). As seen in this study, decrease in weight gain was found in the group treated with AR. This could be explained that the feeding of AR root extract at the given doses for 90 days exhibit estrogen-like effect on rat body weight. Nevertheless, recent study by Chitme, Muchandi and Burli (2009) found that no changes in body weight in animals treated daily for 40 days with AR methanolic extract (50 and 250 mg/kg B.W.) and aqueous extract (50 and 250 mg/kg B.W.) compared to OVX rats. As a result, it is possible that effect of AR root extract in body weight regulating in the OVX rats might be dependent on dosages and duration of treatment.

Effect of AR root extract on reproductive organs

Uterus is a major hormone-responsive reproductive organ. Hormone decline after ovariectomy rapidly regress the organ and weight loss. As expected, our results showed that ovariectomy significantly decreased the uterine weight compared to those of SHAM group, indicating the success of the surgical procedure. Uterine atrophy in OVX rats was absolutely prevented by EE treatment but not by AR root extract. No uterotrophic effect of AR root extract was also evident from decreased uterine weight. Besides, uterine histology and histomorphometry confirmed that AR root extract at the given doses for 90 days had no proliferation.

Just as in the uterus, the mammary glands presented a similar effect in EE-treated rats as comparable to OVX control rats. Proliferative effect is so that increased in area of mammary glands and secretions in ducts confirming the stimulation. EE at 0.1 mg/kg B.W./day seems to have more enhanced effect in reproductive organs. However, the dose of EE used in this study was shown to have the potential to prevent bone loss (Urasopon, et al., 2008). There was no alteration in histology and histomorphometry of mammary gland in AR administration. Therefore, this indicated that no such proliferations were found at two different dose levels of AR treated group.