CHAPTER V

DISCUSSION AND CONCLUSION

The effect of BS on sex hormone levels and reproductive organs in female rats was examined using adult cyclic female rats and treated with 3 different doses of BS. The distilled water (DW) and testosterone propionate (TP) were used as negative and positive control treatments for this study, respectively. We found that serum LH and FSH levels in all BS groups were not different from that in DW group in all 3 treatment period. In TP group, serum LH and FSH levels were significantly decreased when compared to that in DW group. The result suggested that BS had no effect on the secretion of LH and FSH from the pituitary in adult cyclic female rats. FSH activates the granulose cells and causes it to secrete E_2 (Kacsoh, 2000). Because LH and FSH levels were not altered by BS treatment, E_2 levels in BS groups were therefore not different form that in DW group. We known that the chemical constituent of BS is β-sitosterol. β-sitosterol is structurally related to cholesterol, but differed in their side chain configuration. There is a wide variety of phytosterol structures but the most frequent phytosterols in nature are campesterol, β-sitosterol and stigmasterol (Ling and Jones, 1995). The previous studied, in the β-sitosterol treated female rats, there was a transitory decrease in the plasma estradiol concentrations with an increase in the plasma FSH levels (Nieminen *et al.*, 2003). Thus, the amount of β-sitosterol in BS group may be too low to effect on E_2 and FSH level in the cyclic rats. However, the E_2 levels in the TP group were not decreased after treatment, and furthermore, they were the same as that in DW group. TP has an androgenic effect to suppress the secretion of FSH from pituitary and GnRH from

hypothalamus. In general, the decrease of FSH level results in the reduction of E_2 synthesis by granulosa cell (Johnson and Everitt, 1995). These facts indicated that the E_2 levels were maintained in this group not by the secretion from ovary but by the aromatization of TP administered.

The body weights in all BS groups did not differ from those in the DW group whereas the body weights in TP treated group was significantly higher than those in the DW group. An increase in circulating testosterone concentration results in increase in fat-free mass , muscle size (Griggs *et al.*, 2001). In all BS groups were not effect on the weight of ovary and uterus at the end of treatment period. Whereas, exposure to TP significant reduced the weight of ovary, but induced the weight of uterus. It was previously reported that testosterone in vivo can cause a uterine growth as response (Lerner, 1964). However, when organs weights were expressed as the absolute weight, the results were similar to the organ weight in BS and TP treated groups. Furthermore, vaginal smear in rat showed a regular estrous cycle in all BS treated groups, but showed persistent diestrus or leukocyte cells in TP group. In contrast to the non-difference of uterus and ovary weight, a histological observation in 250-BS group showed that the number of uterine glands and endometrium thickness were higher than that of DW group, but did not change in ovarian tissue. Whereas, on TP group showed the number of uterine gland were lower than that on DW group. In the mechanism of the uterine endometrium proliferation, E_2 is a key driver (Johnson and Everitt, 1995). In the present study, the E2 levels were not changed after BS and TP treatment, but the number of uterine glands and endometrium thickness were increased in 250-BS treated groups. It suggested the direct effect of BS on the uterine endometrial proliferation of cyclic female rats, thus the uterine glands were increased.

Complete ovariectomy at diestrus were proved by much lower serum E_2 levels and by much higher serum LH and FSH levels in those ovariectomized rats than that in cyclic rats.

The effect of BS on serum LH, FSH and E_2 levels in ovariectomized rats was not found in 10-BS group. In 50 and 250-BS groups, FSH and E_2 levels were not changed, but LH levels were increased after treatment for 15 days. The effects observed differ from previous result that LH levels tend to decrease due to the 90-day of BS treatment (Bhuntaku, 2000). It may due to the duration of treatment in this study was shorter than that of Bhuntaku (2000). However, serum LH and FSH levels in the TP group were significantly decreased after treatment for 15 days, but the E_2 levels were increased after treatment for 30 days. FSH activates the granulose cells to secrete E_2 and E_2 controls the levels of gonadotropins. The E_2 levels were increased after the administration of TP for 30 days, and the level decreased after the cessation of the treatment for 15 days. Therefore, the E_2 levels in TP treatment group were not considered to be secreted form the ovary, but was supplied by the aromatizative of TP. It suggested that BS at dose of 10 mg/kg.BW/day had effect neither on the suppression of LH nor FSH secretion from pituitary. Whereas, 50 and 250 mg/kg.BW/day of BS were effect to increase the LH levels, but have no effect on the FSH levels. These results show that BS at dose 50 and 250 mg/kg.BW/day may partially have effects on the hypothalamus-pituitary-gonadal axis.

The body weights in all BS and TP groups did not differ from those in the DW group. In all BS groups, there were no effects on the weight of uterus at the end of treatment period. Previous study, β-sitosterol induced significant increases in uterine glycogen concentration and the activity of glucose-6-phosphate dehydrogenase (G6PDH), phosphohexose isomerase (PHI) and total lactate dehydrogenase (LDH) (Malini and

Vanithakumari, 1992). Furthermore, the administration of β-sitosterol to OVX animals significantly increased the uterine weight (Malini and Vanithakumari, 1993). Whereas, the exposure to TP significantly induced the weight of uterus as in cyclic female rat. When the organ weights were expressed as the absolute weights, the results were similar to the organ weight in BS and TP treated groups. No effects of BS and TP were found on vaginal epithelium cells, and they remained to be leukocyte cells throughout experiment as in those found in DW group. This cell type is found in the diestrus stage of estrous cycle in rat in which serum E_2 level is very low. This result indicates that BS at doses of 10, 50 and 250 mg/kg.BW/day did not have enough estrogenic activity on vaginal epithelium cells. Whereas the histological study of uterus showed that in 250- BS group, the number of uterine glands and endometrium thickness were increased. The glands are found in inner layer of uterus or endometrium, and their number increases during proestrus phase in which levels of E_2 is high (Young, 1975). It therefore may conclude that BS at 250 mg/kg.BW/day contains enough quantity of estrogenic-like substances to stimulate the proliferation of uterus, and it may conclude that uterus is more sensitive than vagina in response to estrogenic stimulation.

The most interesting finding of the present study is that 250 mg/kg.BW/day of BS increased the number of uterine gland and endometrium thickness in cyclic female rats, in addition, it was also increased in ovariectomized rats. The present result is explained as that the high dosage of BS (250 mg/kg.BW/day) has effect on the uterine gland and endometrium proliferation. It's effect may due to β-sitosterol in BS. But the uterine weight were not change, it possible that the levels of β-sitosterol in 250-BS were too low to increase the uterine weight.

Even with the highest dose, the BS had no effect on the secretion of LH and FSH from pituitary after treatment for 30 days in normal male rats. However, serum LH and FSH levels in the TP group were significantly decreased after treatment for 15 days. The pituitary derived LH stimulates the Leydig cells to produce T, which in turn passes into the tubules and binds to androgen receptors on the Sertoli cells and thereby supports spermatogenesis (Johnson and Everitt, 1995). In contrast to the non-difference of LH and FSH levels, the T levels were not changed in 10 and 50-BS group after treatment for 30 days. But they were increase in 250-BS group after treatment for 15 days. The increase in the serum T concentration also confirmed to the result of Bhuntaku (2000) studied using the normal male rat. Interestingly, the circulating T levels were the highest in male exposed to β-sitosterol at 5 mg/kg/day. β-sitosterol can be used as precursors of sex steroid biosynthesis (Nieminen *et al.*, 2003). Furthermore, the increase in sex steroid levels can be due to increased synthesis from β-sitosterol precursors (Moghedasian, 2000). The negative feed-back control of T concentration by gonadotropin is well known. The decrease of LH levels causes the testes to shrink, T level to decrease and sperm production to decline (Johnson and Everitt, 1995). It was previous study, in rat, subcutaneous β-sitosterol at 0.5-5 mg/kg/day reduced sperm count and testicular weight (Moghedasian, 2000). Therefore it can conclude that BS has effect on testicular level in normal male rats.

The TP has been known to have an androgenic effect to suppress FSH and LH secretion form pituitary and GnRH secretion form the hypothalamus (Kacsoh, 2000). The TP administration significantly lowered serum LH and FSH levels throughout the study period. We found, however, that the T levels were increased after TP administration throughout the treatment period and decrease after the cessation of treatment, indicating that the T levels detected was that of TP.

The body weights in all BS and TP groups did not differ from those in the DW group. In 10 and 50-BS groups, testis and epididymis weights were not changed when compare to DW group, and then they were expressed as the absolute weight the result were similar to the organ weight. In contrast, exposure to 250-BS significantly increased the seminal vesicle weight at the end of treatment period. Also, when seminal vesicle weights were expressed as the absolute seminal vesicle weights, the increase were also seen. On the previous study, the seminal vesicle was used as indicators of androgenic and /or anti-androgenic activity in vivo, presented similar response profiles, with the seminal vesicles being the most sensitive organs (Stroheker *et al.*, 2003). Some authors have suggested that the seminal vesicles are sensitive organs but there is a wide range of intrinsic variation (Ashby and Lefevre, 2000).After ten days or even after 38 days of treatment, no androgenic effect of the β-sitosterol on the weights of the accessory sex glands was observed, and subcutanous β-sitosterol 0.05-5 mg/kg/day reduced the testicular weight (Moghedasian, 2000). In contrast, exposure to TP significantly increased in both the absolute weights and weights of reproductive organs, including epididymis and seminal vesicle, except testis.

The histology of testes and epididymis in BS groups showed no difference to those in DW group in the process of spermatogenesis. The effect observe differ from previous result treatment in rat, subcutaneous β-sitosterol at 0.5-5 mg/kg/day reduced sperm count (Moghedasian, 2000). The antispermatogenic effect of β-sitosterol observed in rats studied by Malini (1987) as well as by Ghannudi (1978). The histology of seminal vesicle also showed that the folded tubular gland in BS groups were not different from those in DW groups. The TP lessened the absolute weight of testes, though the absolute weight of epididymis and seminal vesicle were increased, the related histological study of seminiferous tubule showed the decrease in the number of spermatozoa, which was

considered to be caused by the suppression of LH and FSH secretion by TP administration. In addition, the reduction on the number of spermatozoa in the epididymis segment appeared due to a reduction in the spermatogenesis. Atrophy of the interstitial cells and a decreased in testis size occur in the rat after treatment with testosterone propionate, the response being attributed to the inhibitory action of the androgen on gonodotrophin secretion (Hunt, 1968). The seminal vesicle also showed a decrease in the thickness of glandular epithelium and seminal vesicle fluid. These phenomena appear to follow the previous observation that although T or LH administered immediately after hypophysectomy can not prevent aspermatogenesis in rats, some reduction in testis size and a reduction in sperm output does occur (Johnson and Everitt, 1995). The antifertility effect of β-sitosterol was pronounced only at the high dose level (5mg/kg/day/rat), but there was a significant decrease in testicular weight and sperm concentrations after long-term treatment with the low dose of β-sitosterol (0.5mg/kg/day/rat). The weights of all accessory sex tissues, except caput epididymis increased following low dose sitosterol treatment. High dose treatment reduced the sperm concentrations as well as the weights, the stop of treatment for 30 days restored only the weight of accessory sex tissues to near normal conditions (Malini and Vanithakumari, 1991).As the development of accessory sex organs depends on T (Kacsoh, 2000), it is concluded that the treatment of 250-BS may affect directly on the seminal vesicle. From the result, T levels were increased in 250-BS group, indicated that, the effect of 250-BS on the weight of seminal vesicle was not androgenic effect.

Complete orchidectomy were proved by much lower serum T levels and by much higher serum LH and FSH levels in orchidectomized rats than that in normal male rats.

The effect of BS on serum FSH and T levels in orchidectomized rats was not found in all 3 treatment period. Serum LH levels in DW and 10-BS abruptly increased after orchidectomy, whereas in 50 and 250-BS groups, the consistent levels were found. However, serum LH and FSH levels in the TP group were significantly decreased after treatment for 15 days, but the T levels were increased since the first day of treatment and throughout the treatment period. It concluded that BS may be able to suppress hypothalamic-pituitary axis. However, the dosages in this study were not enough to show that suppressive effect on the hypothalamic-pituitary axis.

The body weights in all BS and TP groups did not differ from those in the DW group. In all BS groups, the weights of reproductive organs, including epididymis and seminal vesicle at the end of treatment period were not changed. Also, the exposure to TP significant increased the weight of reproductive organs. However, when organs weights were expressed as the absolute weight, the results were similar in all BS and TP groups.

The histology of epididymis and seminal vesicle in BS groups showed no difference to those in DW group. The histology of seminal vesicle showed that the folded tubular gland of seminal vesicle in BS groups were also not different from those in DW groups. The androgenic effect of TP were observed not only in serum FSH and LH levels in orchidectomized rats, but also in the weights and the absolute weights of epididymis and seminal vesicle, on which the histological study showed active state, that is, the pseudostrstified epithelium of ductus and the highly papilla folding pattern of tubular gland at the seminal vesicle. It concluded that the BS influenced neither on the weight and the absolute weight of epididymis and seminal vesicle.

The most interesting finding of the present study is that BS increased the seminal vesicle weight in normal male rats, but not in orchidectomized rats. Similarly, the T levels were increased in 250-BS group of normal male rat, but not in orchidectomized rats. The

present result is explained as that the high dosage of BS has enough androgenic effect to stimulate the seminal vesicle in normal male rats but not enough for the orchidectomized rats. It seems possible that the increased T levels of normal male rats could be caused by the β-sitosterol being used in steroid hormone synthesis.