CHAPTER V

DISCUSSION AND CONCLUSION

In the present study, two doses of PM were used to characterize its estrogenic effects in adult male and female mice on the following reproductive characteristics and parameters: 1) levels of reproductive hormones: LH, FSH, and T (in male) or E_2 (in female), 2) weights of reproductive organs: testes, epididymis and seminal vesicles in male, and ovaries and uterus in females, 3) concentration, viability and motility of sperm collected from the cauda epididymis of male mice, 4) histology of testis in male and uterus and ovary in female, and 5) fertility. The distilled water (DW), and diethylstilbestrol (DES) were used as a negative and positive control treatment for this study, respectively.

In male mice, serum LH, FSH and T levels in all PM groups were not altered. In DES treated group, serum LH, FSH and T were significantly lower at 8 wks of treatment period and recovered within 4 wks after the withdrawal of DES administration. Similar to the finding by Malaivijitnond *et al*. (2004) in rats, the oral treatment with 10- and 100-PM could not affect on serum LH, FSH and T levels in male rat.

Body weights in all PM group did not differ from those in the negative control group, but body weights in DES treated group were significantly higher than those in the negative control group. Exposure to PM significantly reduced weights of epididymis and seminal

vesicle in the 100-PM group at the end of treatment period, but significantly increased weights of seminal vesicle were seen in the 10-PM group at the end of post-treatment period. However, when organs weight were expressed as the relative weights, the decrease in weight of the epididymis in 100-PM group were not statistically significant, but in 10-PM group was increased the weights of testis and epididymis at the end of treatment period and the weights seminal vesicle at the end of post-treatment period. In contrast, exposure to DES significantly reduced in both the relative weights and weights of all reproductive organs, including testes, epididymis and seminal vesicle. Therefore, it can conclude that the treatment of 10-PM did not affect the reproductive organs, but that of 100-PM decreased the weights of epididymis and seminal vesicle in male mice.

All PM treated groups did not show changes in the sperm number. In agreement with the histological examination of testis, it could not be found the difference between all PM groups and negative control group. However, sperm viability and motility were reduced than those in the negative controls in the 100-PM group, and no changes in the 10-PM group. On the other hand, DES treated group showed significant reduction in all of these parameters. The similar and differential responses to estrogen and phytoestrogens exposure on sperm concentration, viability, and motility have been reported previously (Gutendorf and Westendorf, 2001; Benassayag *et al.*, 2002). In adult rats treated orally with ethinyl estradiol at a daily dose of 1 and 10 mg/kg, the sperm number at the tail of epididymis were reduced to almost 50 % of the control group after 1 week of administration. In addition, the percent of motile sperm was significantly reduced in rats treated with ethinyl estradiol after 1 week of treatment at 10 mg/kg BW (Kaneto *et al.*, 1999). Daily oral ingestion of soy extract

containing 40 mg of isoflovanones for 2 months in normal men did not affect on ejaculate volumes, sperm concentration, and sperm motility (Mitcholl *et al.*, 2001). The most interesting finding of the present study is that PM induced the alteration in epididymal sperm viability and motility without changing the sperm concentration.

Both castration and hypophysectomy in rat decrease motility and fertility in epididymal sperm (Dyson and Orgebin-Crist, 1973). The low T level is believed to accelerate sperm transport (Sujarit and Pholpramool, 1985) and thus can explain the reduced number of sperm in epididymis. In the present study, the T levels and sperm numbers were not changed after PM treatment, but sperm viability and motility were reduced in 100-PM group. It suggests the direct effect of PM on the process of spermiogenesis in the seminiferous tubule of male mice, thus the sperm viability and motility were reduced. However, the treatment was not long enough, only 8 wks or 56 days, to observe the effect of PM on spermatogenesis that it takes for 35 days in mice (Clermont, 1972; Johnson and Everitt, 1995) to finish the process, and then changes of sperm number could not be observed. These functional disorders in the epididymis may have resulted from an imbalance between estrogen and testosterone, because spermatogenesis, spermiogenesis and sperm maturation are depended on the delicate balance of the hypothalamo-pituitary- testis axis. These findings indicate that PM could affect the function of spermatogenetic organs before the production of spermatozoa.

The final reproductive outcome examined in male mice was the male 's fertility that is ability to sire offspring in a fixed time period. The treatment with 10-PM had no effect on

fertility in both treatment periods, but the mating efficiency and pregnancy rate was increased after the withdrawal of 100-PM for 4-8 weeks. Male mice treated with DES for 4 weeks completely failed in fertility as shown by the absence of sperm plug and litter, however they could partially recovered within 8 weeks after DES withdrawal. These results clearly suggest that PM treatment for 8 weeks at doses comparable to the human consumption are not effective in reducing male fertility and disturbing the male hypothalamus-pituitary- testis axis. However, the dosage of 100 mg/kg BW/day can promote the fertility after strop feeding.

In female mice, serum LH levels in 10-PM group were higher at 8 wks of treatment and post-treatment period, but the levels in DES and 100-PM treated group were lower than that in the negative control group at 4 and 8 wks of treatment period, respectively. Because of the high variation of FSH levels in DW group, the results of FSH levels after PM and DES treatment were considered only after the comparison to the pretreatment (0 wks) levels. It was found that these were no changes in FSH levels in all PM and DES treated groups. Similar to the finding by Malaivijitnond (2004) in female rats, the administration of PM decreased LH levels more pronounced than FSH levels.

Body weights in all PM treated groups did not differ from those in the negative control group, but those in DES treated group was significantly higher than those in the negative control group. Exposure to PM and DES did not alter of the weights of uterus and ovaries. In addition, when organs weight was expressed as the relative weights, the changes in weight of uterus and ovaries were not seen. Contrary to the result of Chivapat, *et al.*

(2000), the uterus weights in the female rats treated orally with 100- and 1,000-PM were significantly increased when compare to those in the control group. However, vaginal smear in mice showed a significant increase in proliferation and the persistent estrus or cornification in 100-PM and DES groups.

In contrast to the non-difference of uterus and ovary weights, a histological observation in 100-PM and DES groups showed that the uterine endometrium proliferation was much promoted than that in the negative control group as shown by the thicker of endometrium layer, but ovarian folliculogenesis was decreased as shown by the reduction of primary, secondary and graafian follicles. In the mechanism of folliculogenesis, FSH is a key driver (Johnson and Everitt, 1995). It is essential for the final growth of antral follicles and, although it stimulates small growing follicles, it cannot fully control their growth. FSH, together with insulin-like growth factor-I (IGF-I) and estradiol, stimulates the proliferation and differentiation of granulosa cells (Britt and Findlay, 2002). From the results obtained by the present study, the 100-PM and DES treatments keep the FSH level lower than the DW group, the folliculogenesis arrest was therefore occurred. These clearly suggest that the PM affect not only directly on uterus to initiate endometrium proliferation, but also indirectly on folliculogenesis by suppressing the pituitary FSH secretion.

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The final reproductive outcome examined in female mice was the female 's ability to reproduce offspring in a fixed time period. The treatment with 10-PM for 8 weeks increased the mating efficiency and pregnancy rate, but the administration of 100-PM for 4 weeks decreased these parameters, and the 100-PM treatment for 8 wks completely suppressed

mating efficiency and pregnancy rate. The contraceptive influence of PM on female mice could be recovered within 4 weeks after the withdrawal of PM administration. Within the group treated with DES for 4 wks, only one of six female mice had a sperm plug, and that that one mouse could not bear pups and could partially recover at 8 wks after DES withdrawal.

From the result of both sexes, significant decreases in serum LH levels mating efficiency and pregnancy rate were found in female mice treated with 100-PM, but not in male mice. These observations suggest that female mice are more sensitive in response to the PM treatment than those of male mice. Furthermore, it can recommend that the suitable dosage of PM for human use is 10 mg/kg BW, and the males should use the higher dosage if the similar effects are needed. It should be stressed that the recovery from the influence of phytoestorgens is quicklier than that of the synthetic estrogen (DES). Therefore, it can be taken for the one of the beneficial effect of using the medicine herb over synthetic chemicals.

However, the PM treated father or mother could produce the normal offspring and no malformation was found. In addition, there were no significant effects on the relative weights of reproductive organs in both sexes of 50-day pups in all PM and DES treated parents. In agreement with the previous reports on other phytoestrogens, it showed that maternal exposure to genistein (0.1, 0.5, 2.5, 10 mg/kg BW/day) during gestation and lactation did not induce maldevelopment of seminal vesicle and testis weights in male offspring (Fielden *et al.*, 2003). In addition, maternal exposure to 0.4 and 4 mg/kg BW/day of genistein during gestation and lactation did not effect on ovary and uterus in female offspring and testes,

prostate gland and seminal vesicle in male offspring (Kang *et al.*, 2002). Body weights of the pups born from PM and DES treated father or mother during development in the present study are in the normal range (Gad and Chengelis, 1992) and the pups born from PM treated father or mother are not significant different from that of the DW treated-group. Similarly, Kang *et al.* (2002) reported that the maternal exposure to 0.4 and 4 mg/kg/day of genistein during gestation and lactation did not affect on body weight of the offspring during development. These results suggested that the exposure of father and mother mice to PM at doses comparable to the human consumption (10 and 100 mg/kg BW) did not show the adverse effects on the reproductive organs, growth rate and prenatal development (malformation) in offspring, at least in F1. Body weights of the pups during development born from DES-treated mother were significantly higher than that of DW-treated group, it may cause by a less number of litters that they could be delivered by the DES-treated mother than that of the DW-treated mother.

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