CHAPTER V

DISCUSSION AND CONCLUSION

In the experiment of female rats, the basal serum E_2 level (D_1) in cyclic female rats (19.56±7.04 pg/ml) were higher than the OVX rats (0±0 pg/ml). In addition, the basal serum LH and FSH levels (D_1) in cyclic female rats were lower than that of OVX rats (0.18±0.12 and 12.67±1.08 ng/ml for LH and 2.60±0.29 and 31.75±1.93 ng/ml for FSH in cyclic and OVX rats, respectively). The result confirm the absolute removal of ovaries which are the major source of endogenous sex steroid hormones secretion in female rats (Gay and Bogdanove, 1969; Hadley, 2000; Johnson and Everitte, 1995; Kacsoh, 2000; Verjans, van der Molen and Eik-Nes, 1975).

Mc administration to OVX rats could not alleviate the low levels of serum E_2 throughout the treatment period. In OVX rats treated with Mc-10 and Mc-100, LH levels were decreased at D_{31}-D_{46} and the FSH levels were decreased at D_{31}-D_{46} and D_{16}-D_{46}, respectively before returning to the basal levels after the cessation of treatment. In TP treated OVX rats, serum E_2 levels were maintained in the low levels throughout the treatment period while serum LH and FSH levels during D_{31}-D_{46} were decreased to D_{-14} and pre-treatment levels (D_1), respectively. The decrements of LH and FSH levels were still remained after the cessation of treatment when compared to that of DW group. The results of TP in this study were similar to the previous reports (Gay and Bogdanove,1969; Ramirez and McCann, 1965; Swerdloff and Walsh, 1973; Wierman *et al.*, 1990).

Mc treated OVX rats showed no changes on weight and absolute weight of uteri and the uterine histology when compared to the DW treated OVX rats. In addition, the endometrial mucosa of Mc treated OVX rats were thin with the appearance of few numbers and narrow lumen endometrial glands. The degeneration of uterus resulted from the E_2 withdrawal after the removal of ovaries (Johnson and Everitt, 1995). In TP treated OVX rats, uterine weight and its absolute weight were found increased as similar to the previous reports (Garcia and Rochefort, 1977; Ruth and Ruth, 1975; Schmidt *et al.*, 1976). The increase of uterine weight of TP administration resulted endometrial thickness, transformed of epithelial cell shape, increased number and diameter of endometrial glands. The uterine morphology of OVX rats were partially recovered after TP withdrawal for 15 days. It may be due to the slow elimination and high dosage of TP accumulation in rats (Beyer *et al.*, 1974).

After ovariectomy, vaginal smear of all groups showed the complete leukocytes cells that due to the low levels of E_2. Mc and TP administrations in OVX rats did not affect the cell type of vaginal smear. The results may interprete that all doses of Mc and TP did not create enough estrogenic activity to stimulate vaginal proliferation in OVX rats (Johnson and Everitte, 1995; Norris, 1997).

As regards to the experiment of cyclic female rats, serum E_2 levels of Mc-100 treated rats at D_{16}-D_{31} and serum E_2 of TP at D_{31} were increased while serum LH and FSH levels of Mc-100 treated rats at the same day did not change. In addition, serum E_2, LH and FSH levels of Mc-100 and TP at D_{47} were no differences. It may conclude that Mc-100 and TP could increase serum E_2 levels, that increase of serum E_2 levels

were not high enough to promote the positive feedback effect on the hypothalamus and pituitary.

Uterine weight and ovarian weight in all Mc treated rats in cyclic female rats were similar to DW treated rats, except the decreased uterine weight found in Mc-10 group. In addition, there were no changes of uterine and ovarian histology of all Mc treated groups in cyclic female rats. Thus, the decrement of uterine wight of Mc-10 were not considered to be toxic. Moreover, Mc stimulated corpus lutea formation in the ovaries, but it could not answer why Mc could do this effect in this study. In TP treated cyclic female rats, the ovarian weight and its absolute weight were decreased. It was resulted from the suppression of TP to FSH secretion from pituitary gland, resulting in decrease of follicle development and the ovarian weights were decreased thereafter. Moreover, TP administration resulted in increasing of uterine weight and its absolute weight as reported previously (Garcia and Rochefort, 1977; Ruth and Ruth, 1975; Schmidt *et al.*, 1976). TP could induce the endometrium thickness, increased numbers and diameter of endometrial glands. The uterine structure of normal female rats were partially recovered after TP withdrawal for 15 days, may be due to the slow elimination and high dosage of TP accumulation in rats (Beyer *et al.*, 1974).

Regards to estrous cycles, the cyclic female rats showed regular estrous cycles after Mc administration. There were no significant differences of the estrous cycle length between Mc treated groups and DW group in cyclic female rats. Since no estrous cycles occurred after TP administration for approximately 1 estrous cycle in cyclic female rats, it revealed that TP suppressed FSH levels and consequently the E_2

levels. However, the decreased serum E_2 levels by the manner were not caused any significant differences compared to the pre-treatment levels. Because the consistent serum E_2 levels were maintained by 2 pathways; the reduction through the FSH-E_2 axis and the increase by aromatization of TP to E_2.

According to changes of uteri and vagina after TP administration, it could conclude that TP could be aromatized to E_2 and subsequently activates the uterine tissue and weight, but the serum E_2 levels were not high enough to stimulate vaginal proliferation (Aronso, 2002; Beyer *et al.*, 1974)

In the experiment of male rats, the basal serum T level (D_1) in normal male rats (628.97$\pm$183.47 pg/ml) were higher than that of ODX rats (21.03$\pm$13.20 pg/ml). In addition, the basal serum LH and FSH levels (D_1) in normal male rats were lower than that of ODX rats (0.46$\pm$0.09 and 11.63$\pm$0.16 ng/ml for LH and 6.06$\pm$0.31 and 27.15$\pm$0.28 ng/ml for FSH in normal and ODX rats, respectively). The results confirm the absolute removal of testes which are the major source of endogenous sex steroid hormones in male rats (Gay and Bogdanove, 1969; Hadley, 2000; Johnson and Everitte, 1995; Kacsoh, 2000; Verjans, van der Molen and Eik-Nes, 1975).

Mc administration to ODX rats could not alleviate the low levels of serum T throughout the treatment period while serum LH and FSH of Mc of ODX rats were increased. In TP treated ODX rats, serum T levels were increased since the first day of TP administration (D_{16}) and until the last day of study period (D_{62}) similar to the previous reports (Gay and Bogdanove, 1969; Verjans, van der Molen and Eik-Nes, 1975). Although, TP could not alter the serum LH and FSH levels in D_{16}, but serum

LH and FSH levels were decreased to the D_{-14} levels since TP administration for 15 days (D_{31}-D_{46}) in treatment period. Thus, the TP suppressed LH and FSH secretions from the pituitary gland via the negative feedback mechanism. The decrement of LH levels were still remained after the cessation of treatment, but the decrement of FSH levels were still remained only in the first day of post-treatment period (D_{47}), which was similar to the previous reports (Gay and Bogdanove,1969; Ramirez and McCann, 1965; Swerdloff and Walsh, 1973; Wierman *et al.*, 1990).

The administration of Mc to ODX rats alter neither the weights and absolute weights of epididymis and seminal vesicle nor the histology of these organs. Although, Mc could not affect the histology of epididymis and seminal vesicle, but the diameter of epididymal tubules became narrow, the thickness of epididymal epithelial cell lining was found increased with low folded papillary pattern of epithelial cells of seminal vesicle. The results confirm the removal of testes, the major source of endogenous sex steroid hormones in male rats (Johnson and Everitte, 1995). TP administration of ODX rats could increase the weights and absolute weights of epididymis and seminal vesicle similar to the previous results (Gay and Bogdanove, 1969; Moulton and Leonard, 1969, Swerdloff and Walsh, 1973). In addition, the changes of epididymis and seminal vesicle histology were related to the increment of serum T levels throughout the study periods and were partially recovered after the cessation of treatment (Kjell, Tveter and Kjaerheim, 1973). It might be due to the fact that seminal vesicle was sensitive to serum T levels than epididymis (Kjell, Tveter and Kjaerheim, 1973).

In the experiment of normal male rats, serum T, LH and FSH levels of Mc treated groups were similar to the DW treated group throughout the study periods. Serum T levels of TP in normal male rats were increased throughout the treatment period and the T levels of TP in normal male rats were decreased after the cessation of treatment. Serum LH and FSH levels were decreased to the D_1 levels since TP administration for 15 days (D_{31}-D_{46}) in treatment period. The decrement of LH levels were still remained after the cessation of treatment, but the decrement of FSH levels were increased to the pre-treatment levels after the cessation of treatment similar to the previous studies (Gay and Bogdanove,1969; Ramirez and McCann, 1965; Swerdloff and Walsh, 1973; Wierman *et al.,* 1990).

In this study, only Mc-10 increased the weight of testes and epididymis while the histology of testes, epididymis and seminal vesicle were normally found. The results showed that Mc were not a toxic substance. As regards to the testicular weight and its absolute weight in TP group of normal male rats, T plays the important role in maintainance of the weight of testes because T were increased at the end of treatment period. Also, after TP withdrawal or the end of post-treatment period. The weight of testes was decreased and the T levels were low. Therefore, it could conclude that T and LH play the role in testes, but the effect of T directly to the testes was more potent than throughly the LH-T axis. In addition, the numbers of spermatozoa were increased at the end of treatment of TP, in principally, normal male rats, TP changes the spermatids to spermatozoa or so-called spermiogenesis (Johnson and Everitte, 1995; Norris, 1997). TP administration of normal male rats could increase the weights and absolute weights of epididymis and seminal vesicle as the previous studies did (Gay and Bogdanove, 1969; Moulton and Leonard, 1969, Swerdloff and Walsh, 1973).

In addition, the changes of epididymis and seminal vesicle structures were found related to the increment of serum T levels throughout the study periods and partially recovered after the cessation of TP administration (Kjell, Tveter and Kjaerheim, 1973).

According to the weights and histology of testes, epididymis and seminal vesicle of TP group in normal male rats and ODX rats, it may imply that epididymis and seminal vesicle were responsed to TP more quicklier than testes. In addition, seminal vesicle was sensitive to TP more than epididymis in normal male rats and vice versa in the ODX rats.

As regards to the hormonal levels of TP, it was found that serum T levels of normal male and ODX rats were increased since the first day of TP administration which the blood collection for T determination has been done 1 hour after injection. However, serum LH and FSH levels were decreased after TP treatment for 15 days. It can conclude that TP could release from the oily form of injected TP into the bloodstream within 1 hour after TP administration and cross-react with the antibody used for RIA system of T determination. According to the decrement of serum LH and FSH levels of TP treated normal male, ODX and OVX rats, it was found that serum LH levels were decreased greater than the FSH levels and only FSH levels were recovered after TP withdrawal. The results confirm that T or E_2 is not the only hormone that suppress serum FSH levels. The process needs the co-opearation of inhibin to decrease serum FSH levels to the basal values, but the suppression of LH levels needs only T or E_2 (Gay and Bogdanove, 1969; Hadley, 2000; Johnson and Everitte, 1995; Verjans, van der Molen and Eik-Nes, 1975).

According to the results of all experiments, it is concluded that Mc exhibited no estrogenic effects as it could not induce any changes in vaginal epithelium, uterine weight and its histology in OVX rats. In addition, Mc does not show the androgenic effects as it could not induce any alterations in the weights of epididymis and seminal vesicle in ODX rats. Although, Mc are not shown the androgenic effects in this study, but there is an interesting point why Mc could suppress serum LH and FSH levels in OVX rats. The results may be due to the effects of its chemical constituents or metabolites or other factors on LH and FSH syntheses and secretions from the gonadotrophs of pituitary. However, it can not be identified the suppressive effects of Mc on serum LH and FSH in this study. To clarify this question the longer time or the higher dose of treatment of Mc should be used, it is therefore an interesting point for the future study.

Although, there was a strong belief that Mc could exhibit the androgenic effects in Thai folklore story (Suntara, 1931), but Mc-10 group in this study, which was the ordinary dosage used in Thai folklore remedy did not create the androgenic effects. Therefore, the androgenic effects of Mc that were believed to improve the sexual functions in Thai folklore remedy may be due to the psychic condition of users. One explanation is that the believable of users on the androgenic activity of Mc could make no stress of the patient and subsequently improve the reproductive function. Because stress can increase cortisol levels, and then the cortisol can disturb the hypothalamic-pituitary-gonadal axis. Cortisol is regulated by the hypothalamic-pituitary-adrenal axis or CRH-ACTH-cortisol axis, stress related inhibition of GnRH secretion via proopiomelanocortin-positive interneurons in the medial basal

hypothalamus by proopiomelanocortin (POMC), which is fragmented into the ACTH. POMC neurons also fragmented into the endogenous opioid β-endorphin, a well-established antigonadal substances through the GnRH suppression (Johnson and Everitt, 1995; Kacsoh, 2000).

The previous report showed that 400 mg/kg/day of Mc, which is 4 times higher than the dosage used in the present study, increased testicular weight and increased sperm numbers in cauda epididymis and testes (Wuteeraphon *et al.,* 2001). Thus, it is interesting to repeat the present study using the higher dosage of Mc, at least 400 mg/kg/day, to ensure if Mc has the androgenic activity. By the way, at present, there are 2 reports of Mc and reproductive function in men that Mc could exert the effects directly to the sexual functions such as penile erection via vasodilatation in the penis resulted from the inhibition of cAMP phosphodiesterase enzyme of the chemical substances of the stem of Mc such as quercetin, kaempferol and hopeaphenol (Roengsamran *et al.*, 2001; Roengsamran *et al.*, 2004).

Although, the dosages of Mc used in this study did not effect gonadal functions via hormonal system, but Mc may be exert the effects via the autonomic or somatic nervous systems, which the systems could play a role in controlling the gonadal function such as penile erection, or the enzymes link nerve innervation (Benson, 1994). Thus, it is of interest to clarify this aspect in the future.

Nowadays, there are a lot of numbers of men who used some drugs and herbs for such as viagra, Mc and *Butea superba*, Roxb. to prolong the penile erection (Cherdshewasart and Nimskul, 2003; Roengsamran *et al.*, 2001; Roengsamran *et al.*,

2004), but there are a few study on Mc. Thus, it is necessary to gain the basic knowledge of Mc by researching more in various aspects such as the toxicological studies for the safety and applicable dose in human.

Final conclusions

1. Mc does not exhibit the androgenic effects in this study.
2. Mc does not exhibit the estrogenic effects in this study.
3. The dosages of Mc administration in this study are not toxic.