CHAPTER VI

GENERAL DISCUSSION AND SUMMARY

Based on the WHO estimation, 65% to 80% of the world's population relies on traditional medicine as their primary form of health care (Manyam and Sanchez-Ramos, 1999). Plants continue to provide new drugs for treating various diseases before the actual active constituent being chemically synthesized. The findings of toxicologic investigations are influential and decisive for regulation of chemical hazards. Animal toxicity screening has played an important role in the FDA's evaluation of new human drugs (Merrill, 2001). The possibility that *Mucuna macrocarpa*, a plant used in traditional remedy for treating male sexual dysfunction and containing several hormonally active chemicals, may be developed and used as natural medicine leads to an urgent requirement of toxicity study on this plant product. This thesis is the first to report the *in vivo* acute and subchronic toxicity of *M. macrocarpa* crude extract on fish model and provides information for evaluation of the plant reproductive effects. The effects on gonadal structure and function of the Nile tilapia (*Oreochromis niloticus*) were studied. The endpoints for the effects on gonadal structure were determined by histological and ultrastructural biomarkers whereas the endpoints for gonadal steroidogenic function were determined by histochemical and ultrastructural biomarkers.

Acute toxicity test in the tilapia revealed the LC_{50} at 96 hours of *M. macrocarpa* crude extract at 65.72 ppm. This value indicates high toxicity of this plant extract (Matsumura, 1985), and harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment (EC, 2001). The safe concentration below 34.87 ppm,

and the maximum acceptable toxicant concentration (MATC) estimated at 30 ppm may be considered to be a safety guideline for the use of this plant extract in aquatic environment. The histological study revealed histopathological alterations in the livers of the fish exposed to the plant extract in concentration-related manner. The major lesions are large fat droplets accumulation and necrosis, both pyknosis and karyolysis, which indicated high hepatic toxicity of the plant extract on the fish. The mortality responses, the LC_{50} at 96 hours and the histopathological alterations of liver tissues of the treated fish clearly indicate the acute toxicity of *M. macrocarpa* crude extract.

The results from subchronic treatment on the tilapia showed several effects of the crude extract on testicular structure and function. Testicular development based on gonadosomatic index showed significant differences in the early exposure period. The GSI of the treated fish were significantly higher than that of the control ($p<0.05$) at 4 and 5 months post-exposure and then became similar between groups at 6 and 7 months post-exposure. Although the period of high GSI correlated well with the occurrence of complete spermatogenesis in the control fish as discussed in Chapter III, this correlation was not applied to the treated fish. The increased GSI in the treated fish may be the result of histopathological alterations rather than the enhanced testicular development. According to histological and ultrastructural results, *M. macrocarpa* crude extract potentially induced some pathological alterations of testicular tissues in the tilapia. Increase in germ cell apoptosis was observed and may be involved with the properties of quercetin, a constituent in the plant extract. Although the spermatogenesis was comparatively delayed, it was not entirely inhibited. The ultrastructure of germ cells in the testis that were capable of complete spermatogenesis was not altered from the treatment of the plant extract. Histochemical detection of 3β-HSD activity revealed

differences in testicular steroidogenic function between the control and the treated groups. In correlation with the result from histological observation, the activity of 3β-HSD in the treated group was low at 4 months post-exposure and peaked at 5 months post-exposure, then dropped to the levels similar to that of the control in the later period. The results indicate that testicular steroidogenesis may be delayed and/or suppressed by the treatment of the plant extract in early period, and then corrected to the level similar to that of the control in later periods. Ultrastructural features of steroidogenesis found in the tilapia Leydig cell were similar in both control and treated groups. As discussed in Chapter V, the ultrastructural study may provide a more sensitive detection of steroidogenesis in the tilapia testis than the histochemical method. So far, there is no evidence of an enhanced reproductive effect of this plant extract on the male tilapia.

Subchronic toxicity study revealed the effects of the crude extract from *M. macrocarpa* on ovarian structure and function of the tilapia. Ovarian development based on GSI was not significantly different between the control and the treated groups. The results showed some histopathological alterations of ovarian tissues in the treated fish. Apoptosis of oogonia was observed in the immature ovary. The incidences that may be considered as major histopathological effects were observed at ultrastructural level in the follicular cells including an ultrastructural alteration in thecal cells of the cortical alveolar oocytes and hypertrophy of granulosa cells of the vitellogenic oocytes. However, the oogenic process was not altered from the treatment of the plant extract. Ovarian steroidogenesis was studied using histochemical and ultrastructural markers. Similar to the males, the treated ovaries also showed a suppressive activity at 4 months post-exposure and a corrected activity at 5 months post-exposure, and became similar to the control in later period. The activity of 3β-HSD which was found specific to the

thecal cells was correlated with the ultrastructural features of active steroidogenesis found in these cells. It is of importance to note some atrophied thecal cells with the presence of large electron-dense vesicles, dilated endoplasmic reticulum and myelin figures in the treated fish at previtellogenic stage, which may indicate an alteration in steroidogenic function of the thecal cells. It implies that ovarian steroidogenesis in previtellogenic stage may be altered by the treatment of the plant extract.

Difficulty in extrapolation of animal data to humans is mainly due to differences in toxicokinetics, especially biotransformation. Therefore good prediction can be obtained from a number of studies in multiple species and well-validated models (Thomas and Thomas, 2001). Among non-mammalian vertebrates, fish is closer to mammals in the activation and detoxication pathways as well as in mode of toxic action (Hodgson and Levi, 2000). The results from the present fish toxicity study provide reference values and new insights into the long-term effects of this plant extract which will be useful for further toxicological study on the reproductive effects of this plant.