CHAPTER V

DISCUSSIONS AND CONCLUSIONS

The results of this study showed that three extracts of *Passiflora foetida* including PF002-(1-4), PF002-(5-6) and PF003-1 had no significant inotropic and chronotropic effects on isolated rat atria. Another extracts including PF002-7, PF003-2 and PF003-(3-5) caused transiently negative chronotropic and inotropic actions in the first minute. However, the effects of PF003-2 inversely shifted into positive within 10 -15 minute in the left atria. In addition, the negative chronotropic action of PF003-2 on right atria was not prolonged. The negative chronotropic effects of PF003-(3-5) were prominent and sustainable for 15 minute, suggesting its potential effects on pacemaker activity. It is possible that PF003-(3-5) fraction may cause arrhythmia and electrochemical disturbance.

Extracts of *P. foetida* have shown their binding affinity to D_1 and 5-HT_{1A} receptors isolated from rat brains (Wijagkanalan, 2005). In addition, PF003-2 elicited its intrinsic positive inotropic effect with less potential arrythmogenesis. It is very interesting to examine the mechanism of action of this particular extract.

The activation of β-adrenoceptor has been well established for the positive inotropic effects. It is very possible that PF003-2 exerts its positive inotropic action via activation of β-adrenoceptor. In this study, treatment of propranolol only blocked the effect of PF003-2 partially. Hence, it is likely that PF003-2 may exerts positive inotropic

action on isolated rat atria via direct activation of β-adrenoceptor as well as other β-adrenoceptor independent mechanism.

Several other mechanisms other than activation of β-adrenoceptor induce positive inotropic and chronotropic activities. The sympathetic drugs such as norepinephrine or epinephrine may also activate serotonin receptor on the heart (Aloyo and Walker, 1988). Several compounds such as tyramine may exert its possible inotropic effects by stimulation the release of endogenous catecholamines (Battery and Newman, 1964; Fawaz and Simaan, 1964). A number of compounds such as phenylthylamine had both direct and indirect actions on the heart function. The effects of compound on catecholamine storage can be determined with the use of reserpinzed rats (Caughey and Kirshner, 1987). The experiment design was based on the reserpine-induced depletion of NE from its internal storage, leading to the unresponsiveness of the reserpinized tissue toward tyramine treatment (Vincenzi, 2004). In this study, the positive effect of tyramine were not seen in cardiac tissue isolated from reserpinized rats, suggesting that the experimental model was appropriate for determining the positive inotropic effect of PF003-2 on the storage catecholamine. The extract of PF003-2 still elicited the positive inotropic effects in reserpinized atria. Thus, the effects of this extract were not related to catecholamine storage.

Another possibility for inotropic action was activation of calcium release from sarcoplasmic recticulum (SR) which is an internal storage of Ca^{2+} (Klabunde, 2007). The experimental design in the study was modified the method from the study of the

Ca^{2+} handling function in SR of rat ventricular papillary muscle (Yamoto et al., 1996). Caffeine, which is known for its activity to release Ca^{2+} from SR, was used for method validation and as positive control group. The effect of PF003-2 and caffeine on isolated tissue was significantly different. It is likely that the inotropic effects of PF003-2 may be mediated via other pathways, not involving the release of Ca^{2+} from SR.

PF003-2 may exert its effect by direct activation of β-adrenoceptor and β-adrenoceptor independent pathways. It is possible that the extracts may activate serotonin receptor. The activation of serotonin receptors caused an increase in rate and force of contraction on isolated rat atria (Kaumaan, 2006; Kazung, 2004; Rothman, 2001). In addition, the inotropic activity of serotonin cannot be blocked by propranolol (Jirajariyawej, 1988). It was possible that the positive inotropic effect of PF003-2 was related to the activation of serotonin receptor via 5-HT receptor binding (Wijagkanalan, 2005). In this study, treatment of ketanserin partially blocked the effect of PF003-2. Hence, it is likely that PF003-2 may exerts positive inotropic action on isolated rat atria via direct activation of serotonin receptor as well as other serotonin receptor independent mechanisms.

Interesting, in this study, transiently suppression effect of PF003-2 on isolated rat atria was reduced by propanolol and reserpine. By contrast, this inoropic effect was not reduced by ketanserin. Taken together, PF003-2 may exert its positive inotropic action on isolated rat atria via activation of β-adrenoceptor and serotonin receptor. This hypothesis was supported by the evidence that either propranolol or ketanserin (a

serotonin receptor antagonist) blocked the action of serotonin partially. Combination of propranolol and ketanserin completely blocked the positive inotropic effect of PF003-2. The result of this study was in agreement with other observations that combination of propranolol and methylsergide was required to completely block the effect of serotonin. Either propranolol or methlysergide alone suppressed the serotonin activity partially (Jirajariyawej, 1988).

The recent study suggested that antidepressant activity of PF003-2 may be involved direct or indirect binding of serotonin receptor in the CNS (Wijagkanalan, 2005). Regarding to this, it is possible that PF003-2 mediates its antidepressant effect via blocking effect on reuptake of NE or serotonin, like TCA or SSRI (Yildiz et al., 2002, Fernandez et al., 2007). Several drugs with the mechanisms associated with serotonin reuptake have less cardiac effect than those affecting uptake of NE (Jiang et al., 2005). TCA has been well established for its NE-reuptake blockade and its cardiac effects including positive inotropic and chronotropic activities (Thanacoody, 2005, Yildiz et al., 2002). Although the antidepressant mechanisms of PF003-2 are unknown, it is possible that PF003-2 resulted in cardiotoxic action like other antidepressants such as TCA or SSRI (Rodriquez et al., 2001, Roose et al., 1999). The study showed that the positive inotropic action of PF003-2 was unlikely related to a blockade of NE and serotonin reuptake.

It should be noted that *Passiflora foetida* extracts (PF extracts) in this study were a mixture of unknown compounds dissolved with hexane and 90% methanol. Hence,

the results were outcomes of several simultaneous activities from a number of unknown compounds. In order to understanding the mechanism underlying these results, the PF extract should be further purified or characterized. Further research should be carried out to investigate the positive chronotropic and inotropic effects of purified substance in the PF extract.

In conclusions, the results in this study suggested that each of PF extracts were different in their inotropic and chronotropic action on the isolated rat atria. The PF003-2 caused transient depression follow by positive inotropic effect with mild effect pace maker activity. It is possible that PF003-2 may have biphasic response on isolated rat atria. In addition, it is possible that PF003-2 may exerted its positive inotropic effect via activation of β-adrenoceptor and serotonin receptor.