CHAPTER V

DISCUSSION AND CONCLUSION

Study on effective dose

B. superba Roxb. is found in forests in the northern region of Thailand. Phrae and Phayao are provinces locating in the north in which *B. superba* are identified in large amount. The mountain areas of these two provinces are deciduous forest containing large trees. The plants collected from different areas exhibited unequal activities [19]. Therefore, this study was designed to compare the activities of alcoholic extracts of *B. superba* collected from these provinces. The evaluation of the effectiveness of *B. superba* in enhancing penile erection is still difficult. In this study we demonstrated the effect of *B. superba* on electrostimulation-induced erection in the rat model. The intracavernous pressure (ICP) was monitored through electrical cavernous nerve stimulation before and after the administration of *B. superba* extract. An increase in the ICP was employed as a monitoring parameter since *B. superba* has been traditionally used as a rejuvenating in men. In addition, the maximum effective dose was also investigated. The results show that the alcoholic extract of dried root core of *B. superba* collected from Phrae was the most effective with the maximum effective dose of 1 mg/kg BW. It has been reported that *B. superba* collected from different areas possess quite different effects in the erection, on reproductive organ and reproductive behavior of rats [19]. This may be due to the different contents of active components in *B.superba* resulting from the environmental factors such as soil fertility [19].

Penile erection is a result of an increase in ICP. Agents those are capable of increasing blood flow can also enhance the erection of the penis such as nitrovasodialtors (e.g. sodium nitroprusside) [119]. Vasodilators are usually used to rapidly decrease work load to the heart by decreasing blood pressure via dilating of the blood vessels. Therefore, it is possible that any ICP modulating agents are able to alter the MAP. Therefore, the effect of the *B. superba* extracts on the MAP in the rats was

primarily investigated. The result shows that the MAP was not affected by the exposure to the alcoholic extract of dried root core extracts of *B. superba*. This indicates that the alcoholic extracts of *B. superba* may not cause undesirable hypotensive effect.

Regarding the dose response relationship study of the extract from the DRPr, the result exhibited a bell shape response curve. This suggests that *B. superba* extracts may increase the ICP via unknown receptor-mediated mechanisms. Normally, a bell-shape response curve can be observed in a receptor saturation phenomenon. The bell shaped form of the dose response curve is clearly not a consequence of the dependence of simple receptor binding by ligand. It may be that, at the equilibrium, the extracts may bind the receptors at multiple sites. Alcoholic extracts usually contain a number of compounds. At normal concentration of the extract, pharmacological activities of the major compounds are observed while the activities of the others remain silent or unobserved. At higher extract concentrations, the pharmacological activities at those compounds become more pronounced. These activities could be additive, synergism or antagonism to the activities of the major compounds. The antagonistic effect results in reduced activities of those observed at the normal extract concentrations. This phenomenon could happen with the *B. superba* alcoholic extracts since at the higher doses the ICP enhancing activity of *B. superba* were decreased [75].

This study further investigated for possible mechanisms of action of the extract on adenylate cyclase pathway using an *in vitro* model. IBMX is a non-specific phosphodiesterase inhibitor and commonly used to increase the cyclic nucleotide levels *in vitro*. In the presence of IBMX, the endogenous cyclic nucleotides remain unhydrolysed. The result shows that *B. superba* extracts dose dependently relaxed the smooth muscle. Exogenous cGMP and IBMX also relaxed the muscle in dose dependent manner. IBMX or in turn cyclic nucleotides including the exogenous cGMP relax the smooth muscle via cAMP- and cGMP dependent kinases [120]. IBMX non-specifically inhibits phosphodiesterases resulting in increases in the cAMP and cGMP levels. The activity of IBMX is then a result of the activities of both cAMP and cGMP.

Therefore, the extent of the muscle relaxation would be greater with IBMX than that obtained from cGMP alone.

This study further hypothesized that *B. superba* may relax the smooth muscle via the cyclic nucleotide pathways. The relaxation of the smooth muscle in the presence of the extract together with either cGMP or IBMX was investigated. The results show that the extract was able to enhance the activities of cGMP or IBMX observed as the leftward shift of the dose response curves of cGMP or IBMX. This indicates that *B. superba* may act by inhibiting phosphodiesterase enzymes. The evidence that supports this conclusion is that the enhancing effect of *B. superba* was clearly observed when presented with cGMP than when presented with IBMX. This is because cGMP itself does not have inhibiting effect on phosphodiesterase. Therefore, in the presence of the extract the enzyme was inhibited by *B. superba*. Therefore, the relaxation observed were the results of the action of extracellular cGMP and of the unhydrolysed endogenous cyclic nucleotides. In contrast, in the present of IBMX, the phosphodiesterase was already inhibited. The addition of *B. superba* extract could not exert more effects on the enzymes. Only uninhibited enzyme would be suppressed by the extract. This idea is supported by the less increases in the smooth muscle relaxation was observed in the presence of *B. superba* extract and IBMX compared with that observed in the presence of IBMX alone. This is in correspondence with a previous study reporting that *B. superba* inhibits cAMP phosphodiesterase *in vitro* [11]. However, the direction of the shift of the response curve was not clearly demonstrated at all doses of *B. superba* extracts. This may be complicated by the mechanisms of action of *B. superba* itself that appeared the bell-shape. However, it cannot be ruled out that *B. superba* may also act through NO pathways. Further studies are required to clarify the mechanisms of action of *B. superba*. In this study, sildenafil was not including as the positive control. Jeremy et al reported that sildenafil increased endogenous corpus cavernosal smooth muscle cGMP and induced relaxation of the muscles [60].

In conclusion, this study indicates that the extract from dried root core of *B. superba* from Phrae is the most effective among the parts studied with the most effective

dose of 1 mg/kg BW. It is also suggested that *B. superba* may act through cAMP/cGMP pathways.

Toxicity study

Short and long-term treatments of rats and mice with *B. superba* alcoholic extracts revealed no toxicity. The LD_{50} of the extracts obtained from the acute toxicity test is greater than 5,000 mg/kg BW.

Chronic treatment of the animals (0.1, 1 and 10 mg/kg BW) also revealed no mortality during the 6-month observation. Although some hematological parameters in some animals were altered by the treatment, the altered values were not out of the normal ranges and therefore may not possess any clinical significant. No alteration in the liver enzyme functions was observed at all treatment. Chavalittumrong et al reported the findings of significant changes of some hematological, biochemical and histopathological parameter in groups of animals treated with the suspension of *B. superba* at the doses of 100, 250 or 1,000 mg/kg BW/day, respectively [25]. The dilated lumen of epididymidis observed in some male rats and mice may be a result of an increase in the number of spermatozoa [57].

In conclusion, *B. superba* alcoholic extracts produced no acute toxic effect with the LD_{50} of greater than 5,000 mg/kg BW. Chronic exposure to the extract up to 10 mg/kg BW for 6 months did not cause any pathological changes.

Study on male reproductive

Sperm motility has been considered as one of the most important predictors of fertility. Several reports have demonstrated the correlation of motion parameters with fertilization rates [121, 122]. ATP is the main energy source of the sperm motility. A reduced ATP production and/or reduced ATP levels via ATPase hydrolysis result in an insufficient energy and poor sperm motility. *B. superba* increased the sperm motility via unknown mechanisms. It is possible that *B. superba* may increase the ATP production

and/or utilization. In addition, it cannot be ruled out that the plant may act on the NO-cAMP pathway. A number of studies have reported the involvements of the NO and cAMP pathway in the sperm motility. A NOS inhibitor (NG–nitro-L-arginine methyl ester) [123] and a NO scavenger (methylene blue) [124] were reported to inhibit the movement of human spermatozoa [125]. Sildenafil, an inhibitor of phosphodiesterase V, increases the velocity and amplitude in human spermatozoa [126]. Sildenafil also dose-dependently increases cAMP production in the spermatozoa. Human spermatozoa subjected to capacitating conditions increase their endogenous NO synthesis and their intracellular cAMP content. The cAMP synthesis is increased by NO releasing compounds and decreased by NOS inhibitors [123]. These suggest an involvement of the NO and cAMP in the movement of the spermatozoa. *B. superba* has been reported to inhibit phosphodiesterase activity *in vitro* [11]. Therefore, it is possible that *B. superba* may also increase the sperm motility by increasing cAMP production. However, further studies are required to confirm the proposed mechanisms of action of *B. superba*.

An assessment of a sperm motility loss over time is normally used as a quality control of an *in vitro* culture medium used in clinical analysis of Assisted Reproductive Technology (ART). While some laboratories employ sperm survival or stress tests to enhance the predictability of the *in vitro* fertilization rate [127, 128]. The motility results obtained from this study may be useful for further use in clinical study design of *B. superba*. The increased sperm motility in the culture medium by *B. superba* extracts indicates that *B. superba* may be useful in the infertile men.

The number of spermatozoa was increased by *B.superba* extracts. This may be due to β-sitosterol component in *B.superba*. The enzymes in the testis convert β-sitosterol to pregnenolone, an important substrate of testosterone synthesis [129]. The testosterone activates the release of GnRH from hypothalamus. The FSH and LH released by the GnRH induce the spermatogenesis and growth of spermatozoa.