CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

Inflammation is one of the most important health problems, which has been linked to many acute and chronic diseases. Nowadays, inflammatory diseases are also contributing to the increased worldwide morbidity and mortality rates. The treatments against long-term and debilitating effects of inflammatory diseases are necessary (90). NSAIDs are the most widely used agents for anti-inflammatory diseases. They act by inhibiting the synthesis and/or release of mediators and cellular events concerned with the inflammatory responses. Therefore, NSAIDs are usually prescribed for both acute and chronic inflammatory diseases and related symptoms such as pain and fever. Although they are effective but their long-term use is limited by the adverse effects such as gastrointestinal toxicity, water and salt retention, and bone marrow depression. These adverse effects are based largely on findings in many studies of nonselective COX inhibitors or traditional NSAIDs, since they can inhibit the constitutive COX-1, the key enzyme producing house keeping PGs. The adverse effects of NSAIDs lead to the development of new drugs and thereby based on exploiting the beneficial effects of NSAIDs. In this way, the selective inhibition of the inducible COX-2, which its induction occurs during inflammation, has been more directly implicated in ameliorating inflammation. In recent years, the selective COX-2 inhibitors (e.g. celecoxib, etoricoxib, rofecoxib and valdecoxib etc.) has been developed and proved for anti-inflammatory diseases to minimize adverse effects of the traditional NSAIDs (62). However, many studies on adverse effects of selective COX-2 inhibitors have shown an increased risk of cardiovascular and renal adverse events. Therefore, some of the selective COX-2 inhibitors such as valdecoxib and rofecoxib were withdrawn from the market due to these undesired effects (38, 91).

The alternative treatment for inflammatory diseases has been developed and one of the interesting choices is medicinal plants, known as herbal medicines. Herbal

medicines are worldwide used as traditional medicines due to deriving from natural source, being cheap and on the belief of being safe (65-66).

B. siamensis is a plant belongs to Leguminosae family. It is a plant newly discovered in the year 2002 at Phu Miang, Phitsanulok, Thailand. Therefore the information of this plant about phytochemical studies and pharmacological effects have not yet been performed (76). However, there are many studies of pharmacological effects of plants in Bauhinia genus e.g. *B. purpurea*, *B. racemosa, B. tarapotensis*, which possess anti-inflammatory, analgesic and reduced hyperthermia in animal models (73-75). These lines of evidences lead to an interest to investigate the anti-inflammatory, analgesic and antipyretic activities of *B. siamensis* in animal models.

Anti-inflammatory property of BS extract was screened in comparison with reference drug using ear edema formation induced by EPP. The inflammatory mediators released in this model include histamine, 5HT, bradykinin and PGs, respectively. These mediators are orchestrated to promote vasodilatation and increase vascular permeability as well as to produce edema (92). The results of the present study showed that the reference drug, diclofenac (a nonselective COX inhibitor), markedly inhibited ear edema formation. The BS extract also elicited significant inhibitory effect on the ear edema formation induced by EPP. The mechanism of anti-edematous effect of BS extract in this model probably is due to an inhibition of the synthesis and/or the release of the inflammatory mediators of the acute inflammation, especially the products of the COX pathway, i.e. PGs.

Carrageenin-induced rat paw edema formation is one of the widely used model for verification of the ability of anti-inflammatory effect of test compounds (80). This experiment is an excellent test for assessment of COX inhibitors (93). The edema formation induced by carrageenin is mediated by the initial release of histamine and 5-HT followed by the release of bradykinin during the 1-2 h after carrageenin injection (94). The second phase of inflammation is due to the release of PGs which occurs 2-2.5 h after carrageenin injection and lasts about 6 h (80, 95). The release of PGs is closely associated with leucocytes migration to the inflamed site. The presence of PGs, particularly PGE_2, in the inflammatory exudates from the injected foot can be demonstrated at 3 h and periods thereafter (96). It is well established that the second

phase is sensitive to most clinically effective anti-inflammatory drugs such as NSAIDs (95).

The results obtained from the rat paw edema model showed that orally pretreated rats with diclofenac markedly reduced the paw edema induced by carrageenin. Similarly, BS extract significantly reduced the edema formation of the rat paw after edema induction at all assessment times. Base on the inhibitory effect of the BS extract seen at the 3rd h, it suggests that the main mechanism of action probably may be due to the inhibition on the COX pathway. Moreover, the inhibitory effect of the BS extract may partly involve other acute inflammatory mediators e.g. histamine, 5-HT, bradykinin and pro-inflammatory cytokines which are released during the 1st h after carrageenin injection. The results from this model support the possible mechanisms of anti-inflammatory action of BS extract on the COX pathway and on other inflammatory mediators involved in paw edema caused by carrageenin injection.

AA metabolites play a major role on the inflammatory responses. AA metabolites are prostanoids and LTs derived from COX and LOX pathways, respectively. LTs play a significant role in inflammatory reactions as pro-inflammatory mediators. LTC_4, LTD_4, and LTE_4, also known as SRS-A, produce the edema formation whereas LTB_4 causes leukocyte chemotaxis (97-98). Therefore, LOX pathway is another target of anti-inflammatory agents with different mechanisms of action from COX inhibitors. AA-induced paw edema formation in rats can be inhibited by LOX inhibitors (e.g. zileuton), dual inhibitors of AA metabolism (e.g. phenidone) and phospholipase inhibitors (e.g. prednisolone) whereas COX inhibitors are ineffective. This model is useful for investigating anti-inflammatory agents mediated via LOX pathway (81). The results from the present study showed that the BS extract and diclofenac did not exhibit inhibitory activity in AA-induced paw edema formation model. By contrast, prednisolone markedly inhibited paw edema formation in this model.

The results obtained from both paw edema experiments suggest that the mechanism of anti-inflammatory effect of BS extract may be related to inhibition of COX pathway but did not influence LOX pathway.

Cotton pellet-induced granuloma formation in animals is a typical method to provoke chronic inflammatory reactions (99). This method is widely used to assess

the transudative and proliferative components of chronic inflammation (100). The fluid adsorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight correlates well with the amount of granulomatous tissue formed (82, 101). The response to subcutaneously implanted cotton pellets in animals has been divided into three phases including transudative, exudative and proliferative phases. Most anti-inflammatory agents, especially steroids, have inhibitory effect on both transudative and proliferative phases. Swingle and Shideman (1972) found that NSAIDs, such as aspirin, have only slight effect whereas steroids show markedly effective on both transudative and proliferative phases. Anti-inflammatory drugs can reduce transudative weight probably via their ability to inhibit the permeability response of the blood vessels around the cotton pellet implantation. They can also effectively inhibit granuloma formation probably due to interference with proliferative component of inflammatory process (82).

In the cotton pellet-induced granuloma formation, BS extract and diclofenac were slightly but significantly effective in inhibiting the transudative and granuloma formation whereas prednisolone exhibited strong inhibitory effect on both parameters. The results obtained suggest that BS extract suppressed both the vascular permeability and the proliferative components of chronic inflammation.

In cases of body weight gain and thymus weight, it was found that BS extract and diclofenac did not produce any inhibitory effect on both parameters whereas prednisolone markedly reduced the body weight gain and the thymus weight. Steroids, e.g. prednisolone, can prevent or suppress inflammatory reactions. The loss of body weight gain and thymus weight of rats in long-term prednisolone treatment may be due to increased protein catabolism and lymphoid tissue destruction. Steroids can stimulate protein synthesis in the liver and also influence peripheral catabolism of lymphoid and connective tissues, muscle, fat and skin (102). The results obtained suggest a difference in mechanism of anti-inflammatory activity of BS extract and prednisolone, since BS extract did not influence body weight gain and thymus weight. It is therefore postulated that the BS extract does not share these steroidal-like activity.

During chronic inflammation, the recruitment of monocytes and macrophages into inflammatory site occurs. Their main functions are to destroy the invading of foreign materials by phagocytosis process. The phagocytosis process is believed to

contribute to tissue damage by releasing large number of substances include lysosomal enzymes (e.g. phospholipase enzymes) and oxygen radicals. The ability of macrophages to defend tissue homeostasis and participate in inflammatory responses depends on their ability to mobilize granule-membrane proteins and granule content into their external milieu and into phagosomes by regulated secretory processes (103). The elevation of the lysosomal enzymes can be normalized by both NSAIDs and steroid drugs via the stabilization of lysosomal granule membrane as well as suppress the migration of inflammatory cells into the site of inflammation (104). In the present study, BS extract, diclofenac and prednisolone could normalize serum alkaline phosphatase activity to normal level. It is therefore suggested that the effect of BS extract on the alkaline phosphatase activity occurs in a similar fashion as diclofenac and prednisolone by stabilizing the lysosomal membrane.

Inflammatory reaction produces the synthesis and release of the inflammatory mediators, such as histamine, bradykinin, PGs, IL-1 and TNF-α, which their effects are related to pain and fever (105-106). The results obtained from anti-inflammatory models used in the present study indicated that BS extract possessed inhibitory effect on both acute and chronic phases of inflammation, its analgesic and antipyretic effects were thus pursued.

Noxious stimuli produces inflammatory reactions and also induces pain perception. The direct effect of noxious stimuli induced pain perception is the stimulation of nociceptors on afferent nerve fibers and then passing pain signal to the brain. The inhibitory effect of some analgesic agents on the signaling of afferent nerve fibers to brain can reduce pain perception via central pain pathway. However, noxious stimuli do not have only direct effect on nociceptors but also are capable to produce hyperalgesic substances such as histamine, 5-HT, bradykinin, substance P, PGE_2, IL-1 and TNF-α. Hyperalgesic substances produce hyperalgesia by increasing the sensitivity of nociceptors to noxious stimuli and the excitability of the pain perception via the decrease of the threshold of pain signaling (57, 107). Many anti-inflammatoy agents can inhibit hyperalgesic substances by increasing the threshold for activating pain signaling (108).

The writhing response model is used for detecting both central and peripheral analgesia and appropriate for screening the analgesic agents (109). Algesia induced

by an intraperitoneal injection of acetic acid causes liberation of endogenous substances including H^+, K^+, 5-HT, histamine, PGs, bradykinin, substance P and other chemicals that excite pain nerve endings. The writhing response provoked by noxious agent consists of the assembly of contraction of an abdominal wall, pelvic rotation and followed by hind limb extension (57, 86). It has been known that NSAIDs can decrease the number of writhes by inhibiting COX enzyme, the essential enzyme in the synthesis of PGs, in peripheral tissues (110).

In the present study, diclofenac showed significant inhibitory effect on acetic acid-induced writhing response in mice. BS extract also elicited moderately significant inhibition of the number of writhes in this model and its effect showed dose-dependent relation. The anti-inflammatory effect of BS extract appears to involve COX pathway and also probably inhibits other inflammatory mediators such as histamine and bradykinin.

The tail-flick test in rats is an experiment for investigation of analgesic agents mediated via central pain pathway. Algesia in this experiment is induced by thermal radiation using infrared lamp. Thermal application provokes the withdrawal of the tail by a brief vigorous movement, which is known as a tail-flick reaction (109). Heat produces pain by a direct effect on the nociceptors, and then the pain signal is sent to the dorsal horn of the spinal cord and transmitted to higher centers in the brain (111). The opioids are among the most powerful analgesics in clinical use. They act by interfering pain transmission in the CNS (112).

In the tail-flick test in rats, BS extract and diclofenac did not have any effect whereas codeine, an opioid, exerted pronounced inhibitory effect on the tail-flick response. These results suggest that BS extract and diclofenac do not inhibit central nociception. NSAIDs produce analgesic effect by blocking the synthesis of PGs, especially PGE_2 a potent hyperalgesia substance (57) as also proposed for BS extract.

Body temperature is regulated by the thermoregulatory center in anterior hypothalamus, its function is to control the balance between heat production and heat loss. In fever, the set point in thermoregulatory center is elevated (2). Fever may be provoked by many stimuli including bacteria and their endotoxins, viruses, yeasts, protozoa, immune reactions, several hormones and medications. These substances are commonly called exogenous pyrogens. Cells stimulated by exogenous pyrogens form

and produce cytokines called endogenous pyrogens. The most important endogenous pyrogens are IL-1 and TNF-α, which are released into blood circulation then transported to the target cells in the close proximity or in distant sites. These pyrogens produce fever by elevating PGE_2 production and release in the brain. PGE_2 acts on prostaglandin E receptor 3 (EP_3) subtype of PG receptors and centrally affects the thermosensitive neurons in the POA of the anterior hypothalamus, thereby causing the increase the heat production and the decrease the heat loss as well as setting the higher new set point of the body temperature. NSAIDs display antipyretic activity by inhibition of the synthesis and/or release of PGE_2 into the POA of the anterior hypothalamus resulting in decrease of the set point back to normal (2, 113).

Antipyretic activity of BS extract was investigated using yeast-induced hyperthermia in rats (97). Brewer's yeast is one of the exogenous pyrogen that stimulates biosynthesis and release of inflammatory mediators or endogenous pyrogens, especially IL-1 and TNF-α. These mediators can induce expression of PGE_2 which then is transported to POA and generate fever response (58, 89). The results showed that oral treatment with diclofenac strongly reduced the rectal temperature of hyperthermic rats. At the dose of 150 mg/kg, BS extract showed only slight antipyretic effect at 120 min after treatment. The low antipyretic effect of BS extract is perhaps due to its polar property resulting in poor penetration into the CNS. This is in lines with its prominent analgesic effect against peripheral pain but not pain sensation processed in the CNS. However, at the dose of 300 mg/kg, BS extract showed significant reduction of the rectal temperature at all assessment times. The results obtained suggest that light dose of BS extract facilitate the higher penetration into the brain. The antipyretic effect of BS extract is probably related to the reduction of synthesis and/or release of PGE_2 into hypothalamus.

4.2 CONCLUSION

The results obtained in the present study suggest that BS extract possesses anti-inflammatory, analgesic and antipyretic activities. The anti-inflammatory effect of BS extract was found on both acute and chronic phases of inflammation. The anti-inflammatory effect of BS extract was evidenced by the significant reduction of acute inflammatory reaction as proved by ear edema formation induced by EPP application and paw edema formation induced by carrageenin injection. Nevertheless, BS extract did not have inhibitory effect on AA-induced paw edema in rats. The results indicate that the anti-inflammatory effect of BS extract probably mediates via inhibition of COX pathway but not the LOX pathway. Chronic inflammatory study using the cotton pellet-induced granuloma formation showed that BS extract possesses significant inhibition on the transudative and granuloma formation as well as on the serum alkaline phosphatase activity. However, BS extract did not affect the body weight gain and the thymus weight. Therefore, this finding suggests that anti-inflammatory activity of BS extract is free from steriodal-like activity.

The results of analgesic test, using the writhing and tail-flick response, suggest that the BS extract possesses analgesic effect localized in the peripherally via the inhibition of the synthesis and/or the release of hyperalgesic substances. Antipyretic study showed that high dose of BS extract possesses significant reduction of rectal temperature, which probably relates to its inhibition of PG synthesis/release in hypothalamus.

In summary, the results from animal models seem to support the notion that BS extract inhibits PGs biosynthesis and/or release that accounts for its anti-inflammatory, analgesic, and antipyretic effects in the present study.