CHAPTER 4
DISCUSSION AND CONCLUSION

This study is the first report for evaluated pharmacological activities of *S. involucratus*. It revealed anti-inflammatory and analgesic, with no antipyretic activities in various animal models.

The EPP-induced ear edema model was used for screening and evaluating the anti-inflammatory activity of the extract [48]. EPP causes acute inflammatory response by inducing pro-inflammatory mediator releases (e.g., histamine, serotonin, kinins, and PGs) [59] which cause vascular changes, including vasodilatation, and increasing in vascular permeability leading to ear edema formation [5, 59]. The results revealed anti-inflammatory effect of SI extract in this model. Thus, the possible mechanism of action of SI extract may elicit via the inhibitions of the production and/or the activity of these pro-inflammatory mediators.

The carrageenin-induced hind paw edema model was used to evaluate the acute anti-inflammatory activity of the extract. This model is a well-known test that is sensitive to COX inhibitors [49]. The injection of carrageenin into the plantar surface of hind paw causes acute inflammatory response leading to biphasic phase of paw edema. The first phase (0-2.5 h after carrageenin injection) results from concomitant release of histamine, serotonin, and kinins, whereas the second phase (2.5-6 h) is correlated with elevated production of inducible COX-2, PGs, oxygen-derived free radicals, as well as the local neutrophil infiltration and activation [60-65]. In this study, the results indicated that SI extract also possessed anti-inflammatory activity as did a reference NSAID drug, diclofenac. Thus, SI extract may act via the same mechanism(s) of action of diclofenac, i.e. the inhibition of PGs biosynthesis. In addition, it may also inhibit the release and/or the activity of these pro-inflammatory mediators. The AA-induced hind paw edema model was also used to evaluate the anti-inflammatory activity of the extract in acute inflammation. Although, the effect of exogenous AA injection in inflammation is still unclear. It was shown that LOX-inhibitors and steroids, but not COX-inhibitors, are effective in this model [50]. Thus,

this method is widely used for evaluating anti-inflammatory agents which have different mechanism of action from COX-inhibitors. Since SI extract could also inhibit paw edema formation in this model but with lesser inhibitory effect than that was shown in carrageenin-induced hind paw edema model. Therefore, SI extract may also exhibit anti-inflammatory activity via other mechanisms, e.g., LOX-inhibition and/or steroidal-like effect, in addition to its main COX inhibition.

In the cotton pellet-induced granuloma formation model, 7-days subcutaneous cotton pellet implantation can stimulate chronic inflammatory responses. These responses are divided into three phases. The first phase (0-3 h after cotton pellet implantation) is a transudative phase that is defined as the leakage of fluid from blood vessels caused by increasing in vascular permeability. The second phase (3-72 h) is an exudative phase that is defined as the leakage of protein (albumin) from bloodstream around granuloma caused by the intensive maintenance in vascular permeability change. The final phase (3-6 d) is a proliferative phase that is defined as the production of granulomatous tissues caused by continuous pro-inflammatory mediator release [51, 66]. In these chronic inflammatory responses, there is persistence of inflammatory cells leading to the release of pro-inflammatory mediators and oxygen-derived free radicals, as well as lysosomal enzymes such as ALP, which cause subsequent tissue injury. The ALP level is increased and can be detected in serum of animals [5, 52, 66]. Swingle and Shideman (1972) reported that steroids can markedly inhibit effect on both transudative and proliferative phases, whereas NSAIDs can slightly possess these effects. Those drugs can inhibit pro-inflammatory mediator release which is related to increasing in vascular permeability and granuloma formation [51]. In addition, steroids and some NSAIDs, such as indomethacin, aspirin, including diclofenac, are known to possess lysosomal membrane stabilization property [67-70]. Moreover, the long term treatment of steroids can reduce thymus weight and body weight gain. These effects may be due to peripheral catabolism of lymphoid and connective tissues (muscle and skin), and fat [71]. The present results reveal that although SI extract could significantly reduce transudative weight, inhibit granuloma formation, and normalize serum ALP activity to nearly normal level but its effects on transudative weight and granuloma formation were less pronounced when compared to those of diclofenac and prednisolone. In

addition, SI extract did not influence thymus weight and body weight gain. Thus, it seems that SI extract does not possess steroidal-like effect. All these results indicated that SI extract could inhibit chronic inflammation, and its possible mechanisms of action may be partially similar to those of diclofenac.

The International Association of the Study of Pain defined pain as a sensory and emotional experience associated with tissue damage. Generally, pain can be categorized according to both pathologic mechanisms (physiologic and nociceptive). The physiologic pain was defined as rapidly perceives nontraumatic discomfort of very short duration and initiates withdrawal reflexes that prevent tissue injury. On the contrary, the nociceptive pain (inflammatory pain) was defined as noxious perception resulting from cellular damage and pro-inflammatory mediator release which play major roles in its initiation and development [72]. In analgesic tests of this study, both of acetic acid-induced writhing response and tail-flick test models were used for evaluation of nociceptive pain and physiologic pain, respectively.

The acetic acid-induced writhing response model was used for screening and evaluation of analgesic activity of the extract via the inhibitory effect on the writhing response induced by acetic acid. Acetic acid is an irritant which causes the synthesis and release of pro-inflammatory mediators (e.g., bradykinin, serotonin, histamine, PGs, and substance P) that provoke pain nerve endings (nociceptors) [54, 55, 73]. Many scientific studies showed that narcotic drugs (e.g., codeine, morphine, pethidine, and procaine) [74, 75] and analgesic drugs (e.g., aspirin and indomethacin) [74, 76-78] can inhibit writhing response. Thus, this method is used for screening of both centrally and peripherally acting analgesic activity. SI extract could reveal analgesic effect without any clarification of possible mechanism of action in this model.

The tail-flick test model was used in the present study to find out the possible mechanism of action of SI extract via the inhibitory effect on spinal reflex induced by heat. This test is widely used for assessment of analgesic activity through central mechanism at the spinal cord level [79, 80]. The flick of tail is explained by reflex arc in spinal cord which is modulated via descending pathway mechanism [81]. The narcotic drugs such as opioids can almost completely inhibit this reflex [79, 80]. It was found that SI extract exerted less analgesic effect in this test when compared to codeine, whereas in the writhing test, SI extract at the highest dose used in the present

study elicited this effect as effective as diclofenac. These results suggest that the inhibitions of peripheral pro-inflammatory mediator production and/or activity may be the main possible mechanisms of action of SI extract.

The yeast-induced hyperthermia model was used for investigation of antipyretic activity of the extract (via the inhibitory effect on hyperthermia induced by yeast). It was reported that zymosan is a well-known molecule expressed on brewer's yeast (*Saccharomyces cerevisiae*) surface which has been implicated in the recognition of yeast. It mediates an activation of nuclear factor-kappa B (NF-κB) and subsequent secretion of pro-inflammatory mediators (e.g., ROS, TNF-α, IL-8, and IL-12) [82-86]. Several cytokines also increase body temperature, especially IL-1, IL-6, TNF-α, and IFNs, which are called endogenous pyrogens. Both direct and indirect effects of these endogenous pyrogens consist of direct action on the hypothalamus and stimulation of PGE_2 synthesis in the preoptic area of anterior hypothalamus thermoregulatory centers, respectively [5, 10]. The results in this study showed that diclofenac could significantly reduce body temperature, whereas SI extract could not exert this effect. The antipyretic effect of some NSAIDs, including diclofenac results from the inhibition of PGs synthesis within hypothalamus [87]. Since SI extract exerted less analgesic effect in the tail-flick test model which is used for assessment of analgesic activity through central mechanism, therefore it is possible that SI extract revealed no antipyretic effect because it is likely to exert its effects at the peripheral tissue rather than in the central nervous system (CNS).

In conclusion, the results in the present study suggest that SI extract possesses anti-inflammatory and analgesic, but not antipyretic activities. It is preferably effective in acute than chronic inflammation. It possesses analgesic effect mainly via peripheral mechanism. The possible mechanisms of action of SI extract may be due to the inhibitions of the production, release, and/or the activity of several pro-inflammatory mediators at site of inflammation.