CHAPTER 4
DISCUSSION AND CONCLUSION

4.1 Discussion

Inflammation is a normal and essential response to any noxious stimuli that threatens the host and may vary from a localized response to a generalized response. The resulting inflammation can be summarized as follows: 1) initial injury causing release of inflammatory mediators (e.g., histamine, serotonin, bradykinins, slow-releasing substances of anaphylaxis (SRSA), lysosomal enzymes, lymphokinins, and prostaglandins); 2) vasodilation; 3) increased vascular permeability and exudation; 4) leukocyte migration, chemotaxis, and phagocytosis; and 5) proliferation of connective tissue cells. The most common sources of chemical mediators include neutrophils, basophils, mast cells, platelets, macrophages, and lymphocytes. The etiology of inflammatory and arthritic diseases has received a great deal of recent attention but remains, for the most part, unresolved, hindering the development of new agents that are curative in nature. Currently available drugs relieve the symptoms of the disease but are not curative [63].

Anti-inflammatory agents exert their effects through a spectrum of different modes of action. All NSAIDs and steroids currently available are probably able to modulate more than one mediator or cellular event involved in the inflammatory response [25]. The side effects of the anti-inflammatory drugs are one of the major problems in developing medicine today [26]. Therefore, new anti-inflammatory drugs

lacking or having minimal side effects are being searched for all over the world as alternatives to NSAIDs [27].

The results obtained under the conditions for laboratory testing of anti-inflammatory agents revealed the anti-inflammatory effect of the GW extract on a number of experimental models of inflammation used in this study.

The results of the present study revealed the anti-inflammatory activity of the GW extract on acute phase of inflammation. EPP-induced rat ear edema formation is a useful model for screening and investigating the anti-inflammatory activity of test substances on acute phase of inflammation. The inflammatory mediators released in this model including histamine, serotonin, bradykinin and PGs. These mediators are capable to promote vasodilation and increasing vascular permeability as well as synergistically producing edema [64]. It was found that the GW extract elicited significantly inhibitory effect on the edema formation at all assessment times. Similarly to that of phenylbutazone, a COX-inhibitor showed marked reduction of the ear edema. It is possible that the mechanism of this activity may be due to the inhibition of PGs biosynthesis and/or of the release of other inflammatory mediators of the acute phase of inflammation of the ear edema model.

Carrageenin-induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs, since the relative potency estimates obtained from most drugs tend to reflect clinical experience [54]. The local injection of carrageenin induced inflammatory process in the rat involves three phase by several mediators released in ordinate sequence [65]. An initial phase, during the first 1.5 h, is caused by the release of histamine and serotonin, a second phase is mediated by bradykinin from 1.5 to 2.5 h and finally, a third phase, the mediator of which is

suspected to be PGs occurs from 2.5 to 6 h after carrageenin injection. This third phase appears to be the most interesting compared with the two earlier phases. Thus, the maximal vascular response as determined with leukocyte migration to the inflamed area, also reaches its maximum level in this third phase [66]. The carrageenin-induced hind paw edema in rats is known to be sensitive to COX inhibitors, but not to LOX inhibitors, and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the COX involved in PGs synthesis. It has been demonstrated that the suppression of carrageenin-induced hind paw edema after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents [65]. In the present study diclofenac, a COX-inhibitor, markedly reduced the paw edema after carrageenin injection. Oral pretreatment of animals with the GW extract resulted in a significant inhibition of carrageenin-induced hind paw edema at 3^{rd} and 5^{th} h after carrageenin injection. The significant inhibitory effect of the GW extract on carrageenin-induced paw edema at the third hour, suggests that the main mechanism of action of the GW extract may involve the PGs biosynthesis.

AA-induced paw edema in rats is a widely used method for evaluating the anti-inflammatory activity of LOX inhibitors and other agents with a mechanism of action different from COX inhibitors. It is well known that in AA-induced rat paw edema, the products of LOX pathway of AA metabolism, i.e. leukotrienes (LTs), have an important role and the COX inhibitors show low or no activity. Leukotrienes, i.e. LTC_4, LTD_4 and LTE_4 cause edema together with an increased microvascular permeability. AA-induced paw edema model is sensitive to dual inhibition of AA metabolism (phenidone), LOX inhibitors, and corticosteroids (prednisolone) that

inhibit phospholipase A_2, but insensitive to COX inhibitors (NSAIDs) [55]. The results from the present study showed that prednisolone markedly inhibited AA-induced paw edema, as well as the GW extract exerted significant reduction of the paw edema. However, diclofenac, a COX-inhibitor, did not show any effect in this model. These findings suggest that the mechanism of action of the extract might be mediated partly via the LOX pathway or inhibition of phospholipase A_2. In summary from the findings from both paw edema models it suggests that the mechanism of action of the GW extract may be related to the inhibition of both the COX and the LOX pathways or phospholipase A_2.

Cotton pellet-induced granuloma formation is a typical feature of an established chronic inflammatory reaction and can serve as a subchronic and chronic inflammatory test model for investigation of anti-arthritic substances [67]. This model has been employed to assess the transudative and proliferative components of chronic inflammation. Chronic inflammation is a reaction occurs when the acute response is insufficient to eliminate pro-inflammatory agents. The inflammatory granuloma is a typical feature of eatablished chronic inflammatory reaction [70]. Implanting a foreign body under the skin is used to study the effect of a drug on the proliferative phase. 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. The granuloma formed by day 7 is characterized by the formation of a vascularized fibrous capsule containing fibroblasts and infiltrating mononuclear cells [68]. Most NSAIDs, like diclofenac, possess only slight inhibition on the granuloma formation without any influence on the thymus weight and the body weight gain of the animals. The steroidal drug, on the contrary, exhibits profound reduction of the

granuloma and thymus weight as well as the body weight gain [56]. Prednisolone markedly reduced the thymus weight and the body weight gain when compared with those of the control group. Although corticosteroids, such as prednisolone, stimulate protein synthesis in liver, they have pronounced catabolic effects on lymphoid and connective tissue, muscle, fat and skin. The loss of the body weight gain and the thymus weight in long term prednisolone treatment may be due to protein catabolism and lymphoid tissue destruction, respectively. In the present study the GW extract and diclofenac were slightly effective in inhibiting the transudative phase and proliferative phase of inflammation. Swingle and Shideman (1972) showed that non-steroidal anti-inflammatory agents give only slight inhibition whereas steroidal anti-inflammatory agents have a strong inhibition on both the transudative and proliferative phase. The present study also showed the same result. Moreover, prednisolone and the GW extract markedly reduced the body weight gain and the thymus weight while diclofenac produced no effects. These results revealed the similar in mechanism of anti-inflammatory action of the GW extract and prednisolone. It seems that its anti-inflammatory activity may be related to the steroidal-like activity. During chronic inflammation, leukocytes always migrate to the site of injury. They accumulate at sites of inflammation and release lysosomal enzymes and oxygen radical. It is known that the lysosomal enzymes such as alkaline phosphatase activity in serum and in the exsudate elevate during inflammation. This elevation can be normalized by both NSAIDs and steroidal drugs via the stabilization of lysosomal membrane and inhibition of inflammatory cells into site of inflammation [69]. The activity of serum alkaline phosphatase raised in rats in cotton pellet-induced granuloma model was normalized by the GW extract similarly to prednisolone and diclofenac. This effect of

the GW extract may also result from stabilization of the lysosomal membrane and inhibition of the migration of inflammatory cells into site of inflammation similar to NSAIDs and steroidal drugs.

Garcinia wallichii belongs to Guttiferae plant family, the phytochemical studies of the Guttiferae have shown that xanthones constituents are present. The xanthones in the pericarp are composed of mangostione, α-mangostin, β-mangostin, γ-mangostin, gartinin, and garcinone E [42, 43, 44]. The xanthones, α-and γ -mangostins, are major bioactive compounds found in the fruit hulls of the mangosteen [44, 45]. The biological activities of α- and γ-mangostins have been confirmed to consist of anti-inflammatory activities. Recently, Chen *et al* (2008) demonstrated *in vivo* α-mangostin has more anti-inflammatory activity than γ-mangostin [52]. It is possible that the anti-inflammatory activity of the GW extract may be due to xanthones constituents which have been found in Guttiferae plant family.

In the analgesic test, acetic acid-induced writhing response in mice is used to screen for both peripherally and centrally acting analgesic activity. Acetic acid causes pain by liberating endogeneous substances including serotonin, histamine, prostaglandin, bradykinin and substance P which excite pain nerve endings [71]. Prostaglandins are potent hyperalgesic mediators which modulate multiple sites along nociceptive pathway and enhance both transduction (peripheral sensitizing effect) and transmission (central sensitizing effect) of nociceptive information [72]. The results of this model showed that, diclofenac and various doses of the GW extract both significantly inhibited the number of writhes induced by acetic acid in a dose-related manner thus confirming the analgesic effect of the GW extract. Although the writhing response test is very sensitive it has a poor specificity as an analgesic screening

test [73]. Therefore, the tail-flick test in rats was conducted to confirm and study the possible analgesic mechanism of action of the GW extract. The tail-flick test is widely used to investigate the centrally acting analgesic activity. The tail-flick response appears to be a spinal reflex, which is modulated by a supraspinal inhibitory mechanism [59]. Opioids exert their action by interfering pain transmission in the central nervous system (CNS) [74]. In this study, morphine and various doses of the GW extract both significantly inhibited the tail-flick response in rats, but unlikely diclofenac did not show any effect in this model. It was shown that the GW extract produced anti-nociceptive effect on both acetic acid-induced writhing response in mice and tail-flick test in rats. The mechanism of analgesic activity may be via an inhibition of both peripherally and centrally mediated nociception.

Assessment of the acute toxicity is the first step in the toxicological investigations of an unknown substance. The method used in this study is based on the assumption that the toxicity of the extract investigated is completely unknown and in the purpose to determine the acute toxicity index (LD_{50}) with a minimum number of experimental animals as possible [62]. In this study, the GW extract seems to be nontoxic, since a single administration of the GW extract by oral route at the high dose of 3000 mg/kg did not produce mortality or show any signs of toxicity or changes in general behavior or other physiological activities when compared with those of the control group. On the 15th day, all rats were sacrificed to examine gross pathological changes of internal organs, the result showed that there were no detectable abnormalities and no differences in size and color of the internal organs of rats in the control and the GW extract-treated group.

4.2 Conclusion

The results obtained in the present study suggest that the GW extract possesses anti-inflammatory and analgesic effects. The anti-inflammatory effect of the GW extract was found on both acute and chronic inflammation. Acute anti-inflammatory reaction of GW extract was evidenced by the significant reduction of edema formation in the three animal models including EPP-induced rat ear edema, carrageenin- and AA-induced rat paw edema. It seems that the extract reduces inflammatory reaction by inhibiting both the COX and LOX pathways of AA metabolism or inhibiting phospholipase A_2 and/or the synthesis or the release of other mediators, e.g., histamine, serotonin and bradykinin.

In the chronic inflammation, the GW extract reduced the transudative weight and granuloma formation, which may be due to inhibition of PGs synthesis as well as inhibition of fibroblast proliferation and cells migration to injured tissues. Prednisolone and the GW extract markedly reduced the body weight gain and the thymus weight. These results reveal the similarity in mechanism of anti-inflammatory action of the GW extract and prednisolone. It seems that the anti-inflammatory activity of the GW extract may be related to the steroidal-like activity. The GW extract reduced alkaline phosphatase activity in serum, which may be due to its stabilizing effect on the lysosomal membrane and an inhibition of the migration of inflammatory cells into site of inflammation.

The analgesic activity of the GW extract was profound as shown by the significant reduction of algesic reaction on both acetic acid-induced writhing response in mice and tail-flick test in rats. It is likely that this plant extract possesses an analgesic effect by inhibiting both peripherally and centrally mediated nociception.

The rats received GW extract at the high dose of 3000 mg/kg did not cause mortality or show any signs of toxicity or changes in general behavior and abnormality of the internal organs.