DISCUSSION

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair (Vane and Bolting, 1995). It is a complex process and various mediators, e.g. prostaglandins, leukotrienes, platelet activating factor etc. have been reported to be involved in the development of inflammatory diseases (Yesilada *et al.*, 1997). It is also known that anti-inflammatory effects may be elicited by a variety of chemical agents and that there is no remarkable correlation between their pharmacological activity and chemical structure (Sertie *et al.*, 1990). This fact, associated with the complexity of the inflammatory process, makes the use of different experimental models essential when conducting pharmacological trials. On the other hand, the search for a safe anti-inflammatory drug that is free from gastric intolerance continues unabated and a part of such research is the evaluation of medicinal plants known to be used for the treatment of inflammatory disorders (Singh *et al.*, 1989).

The present study on *Garcinia speciosa* was carried out because it is used in folk medicine as an antipyretic agent and some species of *Garcinia* are widely used for different types of inflammatory diseases (Iwu and Anyanwu, 1982; Iwu *et al.*, 1990; Tiangburanatham, 1993; Chairungsrilerd *et al.*, 1996; Likhitwitayawuid *et al.*, 1997). Furthermore, the previous preliminary screening of *G. speciosa* using carrageenin-induced paw edema, showed interesting results for further investigation that it revealed anti-inflammatory activity without ulcerogenic effect (O-urai, 1999).

Of a long list of mediators, including histamine, 5-HT, the kinins, complement, etc., the metabolites of arachidonic acid have become the recent focus of attention. Alone or in appropriate combination, arachidonic

acid products of the cyclooxygenase and lipoxygenase pathways are capable of producing the characteristic signs of inflammation i.e. vasodilation, hyperemia, pain, edema and cellular infiltration (Lewis and Austen, 1981; Issekutz and Movat, 1982). The skin is an organ which exhibits a wide variety of inflammatory reaction and contains significant amounts of both cyclooxygenase and lipoxygenase enzymes (Ruzicka and Printz, 1982; Ziboh *et al.*, 1984). Recently, a skin model of inflammation induced by topical application of various irritants such as EPP or arachidonic acid has been described (Brattsand *et al.*, *1982*; Yong *et al.*, 1984).

EPP-induced ear edema formation is a useful model to investigate the anti-inflammatory activity of test substance on acute phase of inflammation (Brattsand *et al.*, 1982). The inflammatory mediators released in this model include histamine, serotonin, bradykinin and PGs, respectively. These mediators are capable of promoting vasodilatation and increasing vascular permeability as well as synergistically producing edema (Carlson *et al.*, 1985). The results of the present study showed that GS extract exerted a dose-dependent inhibition of ear edema formation induced by EPP. Phenylbutazone, a selective cyclooxygenase inhibitor could markedly reduce the edema of the ear in this model. It is suggested that GS extract probably possessed anti-inflammatory activity, likewise phenylbutazone, by inhibition of the cyclooxygenase pathway and of other inflammatory mediators of the acute phase of inflammation.

Arachidonic acid-induced ear edema is another skin model of inflammation which is useful to screen for compounds showing inhibition on acute inflammatory reaction (Yong *et al.*, 1984). Arachidonic acid produces an intense inflammatory reaction in the mouse ear and subsequent experiments demonstrated that this response can be ameliorated by putative lipoxygenase inhibitors (Chang *et al.*, 1986). Selective cyclooxygenase

inhibitors such as aspirin and phenylbutazone, produce no significant inhibition or are inactive in this model, whereas phenidone, a dual inhibitor of arachidonic acid metabolism shows consistently significant inhibition in this ear edema model (Yong *et al.*, 1984). Thus, it would appear that lipoxygenase products are involved in this model of inflammation and can serve as a suitable model for detecting lipoxygenase inhibitor *in vivo*.

In this experiment the result showed that phenidone, a nonselective inhibitor of arachidonic acid metabolism, exhibited pronounced edema inhibitory effect on arachidonic acid-induced ear edema formation whereas phenylbutazone, a selective cyclooxygenase inhibitor, demonstrated no inhibitory activity on this edema medel. GS extract showed significant inhibition on ear edema formation induced by arachidonic acid. It is therefore possible that GS extract exhibited anti-inflammatory activity in part by inhibition of lipoxygenase pathway.

Carrageenin-induced rat hind paw edema is considered as an acute inflammatory process which is well suited for the comparative bioassay of anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience (Winter *et al.*, 1962). The mediators in carrageenin-induced rat paw edema is reported by Di Rosa *et al.*,(1971). The initial phase of the inflammatory response is mediated by substances found to be histamine, 5-HT and followed by the release of bradykinin during the 1st h after carrageenin injection. The second phase of swelling is due to the release of PGs, occurred 1.5-3 h after carrageenin injection and lasted about 7 h. The significant inhibitory effect of GS extract on carrageenin-induced paw edema at the 3rd h, suggests that the main mechanism of action of GS extract may involve the PG-biosynthesis and/or release. GS extract may also possess some influence on the other mediators e.g. histamine and 5-HT which are released during the 1st h after

carrageenin injection, since it showed pronounced inhibitory activity on the edema formation during this hour as well. The results in this test model support the possibility of mechanism of action of GS extract on the cyclooxygenase pathway and on other inflammatory mediators, which was suggested in ear edema caused by EPP.

The cotton pellet-induced granuloma formation is a typical feature of established chronic inflammatory reaction and can be served as a subchronic and chronic inflammatory test model for investigation of anti-arthritic substances (Spector and Willoughby, 1959). It has been widely employed to assess the transudative and proliferative component of chronic inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlated well with the amount of granulomatous tissue formed (Swingle and Shideman, 1972).

The effect of GS extract on the transudative and the proliferative phase of chronic inflammation was determined. The result showed that GS extract elicited nonsignificant inhibitory activity on the transudative and granulomatous weight whereas indomethacin, a non-steroidal anti-inflammatory drug and prednisolone, a steroidal drug exerted marked inhibitory activity on the formation of transudate and granuloma. It is indicated that GS extract dose not affect the transudative and proliferative phase of chronic inflammation.

When assessment was made on the body weight gain and the thymus weight, it was found that only prednisolone markedly reduced the body weight gain and the thymus weight. Although steroids, particularly corticosteroids such as prednisolone, stimulate protein synthesis in liver, they have pronounced catabolic effects on lymphoid and connective tissue, muscle, fat and skin. The results obtained suggested the difference in

mechanism of anti-inflammatory action of GS extract and prednisolone since GS extract did not influence the body and the thymus weight.

Arachidonic acid metabolites, particularly LTB_4, could mediate or modulate leukocyte influx into inflammatory sites. Leukocytes accumulate at sites of inflammation and are believed to contribute to tissue damage by releasing lysosomal enzymes and toxic oxygen radical (Salmon and Higgs, 1987). The role of lysosomal enzymes such as alkaline phosphatase as mediators of inflammation is well documented (Becker and Henson, 1973). The activity of alkaline phosphatase raised in serum during inflammatory process, results in the damage of tissue and cartilage that can lead to further perpetuation of the inflammation (Bessey *et al.*, 1946). It is known that the lysosomal enzyme activity in serum and in the exudate elevated during inflammation can be normalised by both NSAIDs and steroidal drugs via the stabilization of lysosomal membrane (Salmon and Higgs, 1987). The activity of serum alkaline phosphatase raised in rats in cotton pellet-induced granuloma model in this present study was normalised by all test drugs i.e. prednisolone, indomethacin and GS extract. This result suggests the efficacy of GS extract and reference drugs in protecting the lysosomal membrane system during chronic inflammation. Beside this GS extract was proved in AA-induced ear edema model to inhibit lipoxygenase pathway in producing LTs. This effect, especially by inhibit of LTB_4 biosynthesis, can reduce leukocyte migration into inflammatory area and thereby reducing lysosomal enzyme leakage from those cells.

The analgesic effect of GS extract was also determined by using acetic acid-induced writhing test. Acetic acid causes algesia by liberation of endogenous substances such as PGE_2. PGE_2 is a potent hyperalgesic agent and possesses synergistic effect with histamine and bradykinin which then excite the pain nerve ending in the peritoneal cavity (Salmon and Higgs,

1987). GS extract at doses of 25 – 50 mg/kg was found to exert inhibitory activity on the acetic acid-induced writhing response. This result suggests that GS extract possesses analgesic activity. The anti-inflammatory and analgesic properties of anti-inflammatory drugs in nonsteroidal group are due to their inhibition of PG-biosynthesis (Flower, 1974). The results obtained from many inflammatory models in this study suggest the inhibitory effect of GS extract on PG synthesis. Thus, the analgesic activity of GS extract might also be the result from the inhibition of PG synthesis, similarly to aspirin and other NSAIDs.

Preliminary screening of GS extract found that it did not possess any ulcerogenic activity when orally given in an equal dose of aspirin (O-urai, 1999). It was therefore of interest for further investigation of anti-ulcerogenic effect, as the plant of the same genus, i.e. *G. kola* was found to reduce indomethacin-induced gastric lesion. Therefore, GS extract was further tested in the pylorus ligation and indomethacin-induced gastric lesion models.

The peptic ulcer results from an imbalance between aggressive factors and the maintaining of the mucosal integrity through the endogenous defense mechanism. Aggressive factors are HCl, gastrin, histamine, *H. pylori*, aspirin and other NSAIDs, ethanol, caffeine and stress. Defensive factors are gastric mucus and bicarbonate, gastric mucosal barrier, PGs and mucosal blood flow (Bruton, 1996; Friedmand and Peterson, 1998).

The pylorus ligation model is used for evaluation of anti-secretory activity (Shay *et al.*, 1945). This model causes accumulation of intraluminal HCl. In this present study, cimetidine exerted anti-secretory activity causing significant decreasing of both gastric volume and total acidity. GS extract is found to devoid of anti-secretory activity since it did not decrease gastric volume and total acidity.

NSAIDs, such as indomethacin are known to induce ulcer during the course of their anti-inflammatory action. NSAIDs inhibit cyclooxygenase (COX) enzyme leading to reduction of PG synthesis from arachidonic acid. Two isoforms of COX are COX-1, which is a constitutive enzyme, and COX-2, which is rapidly inducible and responsible for the production of pro-inflammatory PGs (Andrews and Goldman, 1998). High concentration of PGs, especially PGE_2 and PEI_2, are present in the normal gastric and duodenal mucosa and they are responsible for mucous production. Inhibition of PG synthesis by classical NSAIDs, such as indomethacin, which non-selectively inhibit both COX-1 and COX-2, causes gastric and intestinal ulceration and delay gastric ulcer healing in chronic ulcer. Selective COX-2 inhibitors represent a new pharmacological class of NSAIDs with minimal gastrointestinal toxicity. PGs, especially PGE_2 and their analogs inhibit the formation of gastric mucosal necrosis induced by such necrotizing agents including NSAIDs (Robert *et al.*, 1983).

Robert *et al.* (1979) coined the term 'cytoprotective' to describe the property of PGs (and other compounds which have no structural similarity with PGs) by which cells are rendered defensive to stave off gastric mucosal lesions induced by various necrotizing agent, such as ethanol, strong acid or base, and NSAIDs. This pharmacological action is achieved independently of the inhibition of acid and secretion.

GS extract and misoprostal, a PGE_2 analog significantly inhibited gastric ulcer induced by indomethacin. The results suggest that anti-ulcerogenic activity of GS extract is probably associated with increases of defensive factors such as gastric mucus and microvascular blood flow. The results in the present study are similar to those observed with the hexane extract of the plant in the same genus i.e. *G. kola*, which showed anti-inflammatory activity on carrageenin-induced pedal edema model in rats and

inhibited gastric lesion induced by indomethacin (Brade, 1993; Ibironke *et al.*, 1997).

In conclusion, the results of the present study show the anti-inflammatory and analgesic activity of GS extract. The anti-inflammatory effect of GS extract was found prominently on acute inflammation. It is likely that GS extract reduces inflammation by blocking both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. On the chronic inflammation, GS extract did not inhibit the transudative phase and fibroblast proliferation. Moreover, GS extract appeared to be devoid of steroidal like effects such as causing marked decrease of the normal body weight gain and the thymus weight. However, GS extract reduced the alkaline phosphatase activity in serum. The inhibition of lysosomal enzyme (alkaline phosphatase) activity might be due to lysosomal membrane stabilization which may be useful for the prevention the tissue and cartilage from damage in chronic inflammatory diseases. The analgesic activity of GS extract might be mediated by blocking PG-biosynthesis, since PGs, especially PGE_2, are responsible for potentiation of analgesic caused by some mediators i.e. histamine and bradykinin. GS extract has no anti-secretory activity. However, it is likely that GS extract exerted anti-ulcerogenic activity via defensive mechanism. Furthermore, the inhibitory effect of GS extract may be more selective on COX-2 rather than constitutive COX-1. It can be concluded from the present study that the beneficial effects of the methanol extract from the bark of *G. speciosa* on inflammation may arise from its action on the formation and/or release of mediators of inflammation and the stabilization of lysosomes with cytoprotective activity. Further studies both in phytochemistry and pharmacology should be conducted to find out the active principles to be potential candidates as novel safe therapeutic agents

for the treatment of inflammatory diseases that is free from gastric intolerance.