DISCUSSION

Inflammation is a localized, protective response to injury and arises from the resultant cell damage (Gallin and Snyderman, 1999; Benjamini *et al.*, 2000). It involves a complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair (Collins, 1999; Fantone and Ward, 1999). It is a complex process and various mediators e.g. histamine, serotonin, bradykinin, PGs, LTs, IL-1 etc. have been reported to be involved in the development of inflammatory diseases (Guyton and Hall, 2000). Anti-inflammatory agents exert their effects through a spectrum of different modes of action. All NSAIDs and steroids currently available are probably polycomponent in that they are able to modulate more than one mediator or cellular event concerned with the inflammatory response (Furst and Munster, 2001). However, prolonged use of these agents should be avoided due to the severe adverse/side effects. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects (Vane and Botting, 1998; Furst and Munster, 2001). Nowadays, plants have long provided mankind with a main source of new chemical substances with potential therapeutic applicability.

The investigation of "Thao yaai mom" (*C. petasites*) for anti-inflammatory, analgesic and antipyretic activities is relevant because of its therapeutic use in Thai folklore medicine as anti-asthmatic, anti-inflammatory and antipyretic drugs (Mokkhasmit *et al.*, 1976; Pongboonroud, 1976; Tiangburanatham, 1996). An anti-asthmatic activity of the ethanol extract of *C. petasites*, has been proved to be potential by the work of Chatluang (2000). In present time, it is well known that the inflammation of airway wall has been recognized as one of a prominent feature of fatal asthmatic attack. These inflammatory symptoms consist of edema formation, plasma exudation and airway secretion which finally resulting in airway obstruction. Furthermore, the mediators of inflammation e.g. PGs, LTs, PAF, etc. also play role in asthma although the preference of

mediators involved in the two aspects are different (Barnes, 1987). From the close relationship of these two therapeutic effects, it is therefore interesting to investigate the inflammatory activity of the extract from this plant.

Generally, 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).

EPP-induced ear edema formation is a useful model for investigating the anti-inflammatory activity of test substance on acute phase of inflammation (Brattsand *et al.*, 1982). Topical application of EPP produces an increase in the vascular permeability with leukocyte infiltration into the ears. The long-lasting inflammatory response, which provokes by EPP, is associated with a transient increase in PG production. PGs and other inflammatory mediators, which are involved in this model, such as histamine, serotonin and bradykinin, 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 CP extract exerted a dose-dependent inhibition of ear edema formation induced by EPP. Phenylbutazone, a selective COX inhibitor, could markedly reduce the edema of the ear in this model. It is suggested that CP extract probably possessed anti-edematogenic activity, likewise phenylbutazone, by inhibition of the COX pathway and/or of other inflammatory mediators of the acute phase of inflammation.

Carrageenin-induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has frequently used to assess the anti-edematous effect of natural products (Ferrandiz and Alcaraz, 1991). The hind paw edema, which follows intraplantar carrageenin injection, involves a complex and time-dependent

synthesis/release of a plethora of different inflammatory mediators. As previous studies, edema formation due to carrageenin in the rat paw is a biphasic event (Vinegar *et al.*, 1969) and the mediators involved in this model is reported by Di Rosa *et al.*(1971). The initial phase of the inflammatory response is mediated by the substances found to be histamine and serotonin (Crunkhon and Meacock, 1971) and followed by the release of bradykinin (Crunkhon and Meacock, 1971; Di Rosa *et al.*, 1971) during the 1st h after carrageenin injection. The second accelerating phase, which is also the complement dependent reaction, involves the release of PGs, among other substances (Vinegar *et al.*, 1969; Crunkhon and Meacock, 1971) occurs 1.5-3 h after carrageenin injection and lasts about 7 h. It is well established that the second phase (3 h after pedal injection) is sensitive to most clinically effective anti-inflammatory drugs, particularly NSAIDs (Vinegar *et al.*, 1969; Di Rosa *et al.*, 1971). Most reports suggest that NSAIDs preferentially inhibit the second phase response presumably by inhibiting the inducible COX-2 enzyme which is believed to be responsible for the generation of pro-inflammatory PGs in the later stages of this and other inflammatory models (Vane and Botting, 1998). However, it has been reported that part of the PGs produced following carrageenin injection in the foot pad may be also produced by the COX-1 enzyme (Smith *et al.*, 1998). This would be true especially at the early time points before COX-2 protein is present.

In the present work, the profile of carrageenin-induced paw edema is similar to that found by others in being biphasic over early and late periods. Orally pre-treatment of animals with CP extract as well as aspirin resulted in a significant and dose-related inhibition of carrageenin-evoked hind paw edema. Anti-inflammatory intensity elicited by CP extract, however, was weaker than that of aspirin. Regarding the possible mechanisms involved, several inflammatory processes have been suggested to play a role, e.g. activation of complement, and release of histamine, kinins, AA metabolites (particularly PGs) and pro-inflammatory cytokines (Di Rosa *et al.*, 1971; Hirschelmann and Bekemeier, 1981). It is assumed that at least a part of these processes is a subject of inhibition by CP extract. The result in this test model supports the possibility of anti-

inflammatory mechanism of action of CP extract on the COX pathway and/or on other inflammatory mediators.

In order to clarify the mechanism by which CP extract reduced carrageenin-induced paw edema, it was decided to investigate other possible effect of CP extract using AA-induced paw edema model in rat. The choice of AA is based on the knowledge that it is associated with the rapid release (within minutes) of lipoxygenase products (Young *et al.*, 1984; Chang *et al.*, 1986). The involvement of lipoxygenase products, particularly LTs, and mast cell mediators in the edematous response to AA render this model potentially useful for studying anti-inflammatory agents with a mechanism of action different from that of COX inhibitors (DiMartino *et al.*, 1987). Data of previous studies found that selective COX inhibitors produce no significant inhibition or are inactive in this model, whereas dual inhibitors of AA metabolism show consistently significant inhibition of edema (Young *et al.*, 1984; Chang *et al.*, 1986).

The injection of AA into hind paw, produced significant edema after 1 h. CP extract, at all doses that exhibited anti-inflammatory activity in carrageenin-evoked paw edema model, as well as aspirin did not exert any inhibitory activity on the AA-induced paw volume. On the other hand, the dual blocker phenidone markedly inhibited AA-induced paw edema. It is well known that on AA-induced rat paw edema, AA-derivatives, especially LTs, have an important role and the COX inhibitors show low or no activity (DiMartino *et al.*, 1987). Since CP extract did not show any activity in this edema, it seems that its anti-edematogenic activity is not related to the lipoxygenase pathway, but it could be related to the COX pathway and/or the releasing system of other inflammatory mediators.

The difficulty in dealing with the inflammation is its complex and chronic process. To overcome this aspect, many attempts have been made to develop the subchronic and chronic test models. Among them, 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 (Ismail *et al.*, 1997). It has been widely employed to assess the

transudative and proliferative component of chronic inflammation. The fluid adsorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlated well with the amount of granulomatous tissue formed. In addition, there is a general agreement that most anti-inflammatory drugs can effectively inhibit this granuloma formation, probably via their ability to interfere with the proliferative component of the inflammatory process (Swingle and Shideman, 1972).

In the present study, CP extract as well as aspirin elicited significant inhibitory activity on the transudative weight but had no affect on the formation of granuloma. On the contrary, prednisolone, a steroidal anti-inflammatory drug, exhibited profound reduction of transudative weight and granulomatous formation. These results indicate that CP extract has no inhibitory activity on the proliferative phase of chronic inflammatory process. Moreover, prednisolone markedly reduced the body weight gain and the thymus weight while CP extract and aspirin produced no effects. The loss of body weight gain and the thymus weight in long-term prednisolone treatment may be due to protein catabolism and growth inhibition which is a characteristic of the steroidal drugs (Boyd, 1982). 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. Supraphysiologic amounts of corticosteroids lead to decreased muscle and lymphoid mass. These results reveal the difference in mechanism of anti-inflammatory action of CP extract and prednisolone. Anyhow, CP extract could reduce the transudative weight, which suggests its inhibitory activity on the increased permeability of the capillaries.

In order to understand the biochemical mode of action of CP extract, the level of lysosomal enzyme, i.e. alkaline phosphatase in serum, has been studied. During inflammation there is a migration of leukocytes, especially PMNs and monocytes, to the site of injury. Their main functions are to destroy the invading microorganisms and clear the tissue of the dead cells (Salmon and Higgs, 1987). The digestion of ingested material is accomplished by the formation of phagolysosomes through fusion of lysosome with vacuoles surrounding the phagocytosed material and the emptying of

lysosome contents into it. Generally, lisosomes are packed with hydrolytic enzymes and cationic protein (Collins, 1999). When leukocytes phagocytize an inflammatory agent, they release lysosomal hydrolases which damage the surrounding tissues and cartilage that can lead to further perpetuation of the inflammation (Bessey *et al.*, 1946, Weissman *et al.*, 1971). Nevertheless, it is known that the lysosomal enzyme activity in serum and in the exudate elevated during inflammation can be normalized by both NSAIDs and steroidal drugs via the stabilization of lysosomal membrane (Salmon and Higgs, 1987). Naik and Sheth (1978) suggested that drugs capable of preventing the release of lysosomal contents or of antagonizing the effect of the released factors would produce a significant anti-inflammatory effect. In the present study, the activity of serum alkaline phosphatase raised in rats in cotton pellet-induced granuloma model was normalized by CP extract similar to reference drugs, aspirin and prednisolone. This result suggests that one of the possible modes of action of CP extract may be through the stabilization of the lysosomal membrane system, protecting the lysosomes from disruption.

In the present research, CP extract was also tested for their ulcerogenic activity. Interestingly, this extract was found to have no ulcerogenic effect when compared with the reference anti-inflammatory drugs, aspirin and prednisolone. The ulcerogenic potential is a well-known side effect of NSAIDs and it is accepted that it is mediated via inhibition of PG synthesis (Vane and Botting, 1998). High concentration of PGs, especially PGE_2 and PGI_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 aspirin, which non-selectively inhibit both COX-1 and COX-2, causes gastric and intestinal ulceration and delay gastric ulcer healing in chronic ulcer. In recent years, research on the novel drugs to eliminate these side effects has been intensified (Vane and Botting, 1998; Furst and Munster, 2001), and selective COX-2 inhibitors become to represent a new pharmacological class of NSAIDs with minimal gastrointestinal toxicity (Hawkey, 1999; Davies *et al.*, 2000). The above described experiments clearly demonstrate that the anti-inflammatory activity of CP extract may be due to the inhibition of PG synthesis and/or other inflammatory mediators. Hence, the

possibility of the anti-inflammatory effect of CP extract without ulcerogenic effect may be due to PG inhibition by selective action on COX-2, without an important effect on constitutive COX-1. Whatever the mechanism of action may be, the anti-inflammatory without ulcerogenic effect is a clinical desirable characteristic of novel anti-inflammatory agents.

Fever, pain and inflammation are all closely related as part of the body's defense mechanisms to injury (Milton, 1982). NSAIDs, which exert their activity by inhibition of PG biosynthesis, usually possess analgesic and antipyretic activities. It is therefore of interest to investigate analgesic and antipyretic of CP extract as well.

In the writhing response test, acetic acid was injected intraperitoneally into the mice as an inducer for causing writhes. The abdominal constriction response is thought to involve, in part, local peritoneal receptors. Collier *et al.* (1968) proposed that acetic acid acts indirectly by releasing endogenous mediators that stimulate the nociceptive effect but also stimulates neurons that are sensitive to other drugs such as narcotics and centrally acting agents. Acetic acid caused algesia by liberating endogenous substances including H^+, K^+, serotonin, histamine, PGs, bradykinin, sP and many others that excite pain nerve ending (Ferreira and Nakamura, 1979; Salmon and Higgs, 1987). It has long been known that aspirin and other NSAIDs could decrease the number of writhes by inhibiting enzyme COX, the essential enzyme in the synthesis of PGs, in peripheral tissues (Fields, 1987). PGs, particularly PGE_2, are synthesized at the site of injury and can act upon the peripheral afferent terminal to facilitate afferent transduction and augment the inflammatory state (Salmon and Higgs, 1987; Griffith, 1999). In the present work, the analgesic effect of CP extract was also determined by using acetic acid-induced writhing test. Surprisingly, CP extract, orally administered at the effective doses in acute inflammatory experiments, was poor effective in blocking writhing response.

Brewer's yeast-induced hyperthermia in rats was used to investigate antipyretic activity of CP extract. Fever may be provoked by many stimuli. Most often, they are bacteria and their endotoxins, virus, yeasts, protozoa etc. These substances are

commonly called exogenous pyrogen. It is well known that yeast releases high molecular weight lipopolysaccharides, which in turn cause, sustained release of leukocyte pyrogen (also called endogenous pyrogen). The most important endogenous pyrogens are IL-1 and TNF-α. These endogenous pyrogens produce their effects by activating PG synthetase in the hypothalamus and the PGE_2 produced causes a rise in body temperature (Bowman and Rand, 1980). The results obtained in the present study showed that CP extract was found to reduce markedly the pyrexia induced by brewer's yeast. Additionally, the dose required to produce significant effect was generally similarly to that of aspirin. From above inflammatory tests, the anti-inflammatory activity of CP extract was postulated to be mediated through the inhibition of COX pathway and/ or other inflammatory mediators. Therefore, it is logical to presume that the antipyretic effect of CP extract may correlate to some extent with inhibition of the synthesis and release of PGs and/or endogenous pyrogen.

As part of this pharmacological study of the possible anti-inflammatory, analgesic and antipyretic activities of CP extract, Hippocratic screening test of this extract has been performed. A single administration of CP extract by the oral route up to a dose of 5000 mg/kg did not exhibit any mortality or produce any sign of toxicity in rats. There was no observable behavioral or autonomic changes in the animals or no significant changes in daily body or organ weight during the next 7 days when compared with control group. The results of Hippocratic screening test indicated that CP extract is non-toxic.

Overall, on the basis of the results obtained it suggests that CP extract show moderate anti-inflammatory and strong antipyretic properties. The significant reduction in pyrexia after small dose of CP extract administration indicates the potent antipyretic activity of this plant. The effective anti-inflammatory effect of CP extract was evidenced by the significant reduction of edema in two models of acute inflammatory reaction, EPP-induced ear edema and carrageenin-induced paw edema. It should be taken into consideration that the mechanism involved in the genesis of the EPP- and carrageenin-induced edema can cause the release of histamine, serotonin, kinins, and PGs, among

other substances (Crunkhon and Meacock, 1971; Di Rosa *et al.*, 1971). Also, the yeast-induced hyperthermic test implicates the release of PGs and other pro-inflammatory cytokines e.g. IL-1 and TNF-α (Milton, 1982; Martin *et al.*, 1988). Therefore, the anti-inflammatory and antipyretic mechanism of action of CP extract may be related to the interfering with the release system and/or biosynthesis process of PGs, pro-inflammatory cytokines, and many other mediators such as histamine, serotonin and kinins. Considering the above results, unlike NSAIDs, it was surprising to find that CP extract, administered at active doses in acute inflammatory experiments, was ineffective in blocking writhing response. This indicates that CP extract may inhibit different aspects and chemical mediators of inflammation. Nevertheless, it is well known that although all NSAIDs act mainly by inhibiting PG biosynthesis, their relative effectiveness as anti-inflammatory, analgesic and antipyretic agents differs markedly (Heller *et al.*, 1985; Insel, 1999; Furst and Munster, 2001). Ibuprofen is more effective than aspirin for the relief of the pain accompanying dysmenorrhea. Acetaminophen, an effective analgesic and antipyretic agent, is a weak PG inhibitor in peripheral tissues and possesses no significant anti-inflammatory effect. Diflunisal is more potent than aspirin in anti-inflammatory tests in animals and appears to be a competitive inhibitor of COX. However, it is largely devoid of antipyretic effect, perhaps because of poor penetration into the central nervous system (Insel, 1999; Furst and Munster, 2001).

However, the results obtained in this research were similar to the previous results investigated on ginger (*Zingiber officinale* Roscoe) extract. It had been found that the ethanol extract of the rhizomes of ginger reduced carrageenin-induced paw swelling and yeast-induced fever. Although, its anti-inflammatory and antipyretic properties may correlate to some extent with inhibition of release of PGs, as shown in rat peritoneal leukocytes *in vitro* study, but the ginger extract was ineffective in suppressing the writhing response induced by intraperitoneal acetic acid (Mascolo *et al.*, 1989). Likewise the prior research of Kasahara *et al.* (1983), the methanol extract of ginger exhibited negligible analgesic activity, since this extract, only at the high doses (up to 3-10g/kg), showed inhibitory effect on acetic acid-induced writhing response test.

Anyhow, it had been reported that one of the pungent constituents of ginger, shogaol, which is found to have COX inhibitory activity, could be produced an anti-inflammatory, analgesic and antipyretic effects (Murakami *et al.*, 1965; Suekawa *et al.*,1984; 1986). Moreover, Fabry *et al.* (1998) and Shale *et al.* (1999) had suggested that plant extracts which showed moderate or low anti-inflammatory activity in the COX bioassay may have active compounds but probably in smaller amounts and/or the screened crude extracts could yield more potent compounds once they had undergone some purification. Thus, further work is obviously required to fractionate, purify and identify the structure of the active principle(s) present in this extract, as well as to isolate enough pure substance(s) in order to determine their mechanism of action. CP extract is a crude extract, although it showed a certain specificity concerning the inhibition of the different chemically-induced edema, additional studies are necessary to confirm the mechanism of action. Isolation and characterization of the active principle should also be conducted.