DISCUSSION

D. variegata was selected for the present study because of its analgesic reputation and some previously reported biological activity (Mokkhasamit, *et al.*, 1971). To date, however, no work on anti-inflammatory potential of this plant had been done. According to the preliminary study, the crude methanol extract of *D. variegata* possessed significant analgesic activity on writhing response in mice (Table 1). Anyhow at room temperature this crude methanol extract separates into two layers with oily part on the top, it was therefore partitioned with hexane to give two fractions i.e. hexane and methanol fractions.

Both fractions were tested for the analgesic, antipyretic and anti-inflammatory effects. The analgesic activity of the methanol and hexane fractions was evaluated using the writhing response, the tail-flick test and the formalin test. Ear-edema model and yeast-induced hyperthermia in rats were used to assess anti-inflammatory and antipyretic effect, respectively, of both fractions.

The writhing response or the abdominal contraction of the mouse to an intraperitoneal injection of acetic acid or other noxious chemicals is used to screen for both peripherally and

centrally acting analgesic activity. Acetic acid causes algesia by liberating endogenous substances including H^+, K^+, 5-HT, histamine, PGs, bradykinin, sP and many others that excite pain nerve endings (Raj, 1996). The results obtained from the present study showed that the methanol fraction did not possess any analgesic activity on acetic acid-induced writhing response in mice. On the other hand, the hexane fraction exhibited pronounced analgesic activity on this model. The result obtained indicates that the active principle(s) possessing analgesic property is (are) in the hexane fraction which contains nonpolar substances. According to the percent inhibition on the number of writhes obtained with the various doses of hexane fraction, it was found that the intensity of the analgesic effect was similar to that of aspirin at the same doses. Aspirin and other NSAIDs have a broad range of efficacy for symptomatic treatment of pain. They all inhibit cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors (Fields, 1987). The mechanism of analgesic action of the hexane fraction could probably be due to the blockade of the effect or the release of endogenous substances that excite pain nerve ending similarly to aspirin and NSAIDs.

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 supraspinal inhibitory mechanism. The morphine-like analgesics, which directly act on the reflex arc and through the inhibitory supraspinal mechanism, exhibited the inhibitory effect on tail-flick response (Harris, *et al.*, 1969). In this present study only morphine, an opioid and a centrally acting analgesic drug, could produce a 100% inhibition on the tail-flick reflex. Aspirin possessed slight analgesic effect on this tail-flick response. Similarly, the hexane fraction exhibited only slight inhibition on the tail-flick reflex with the percentage of inhibition of 21. Methanol fraction did not show any analgesic effect. The results obtained from both tests rather suggest that the hexane fraction and aspirin seem to possess similar intensity of analgesic effect which is mostly mediated via a peripheral mechanism by inhibiting the PGs-mediated potential of algesic action of bradykinin (Nakamura, *et al.*, 1986). Hexane fraction also could partly act via the central nervous system in producing analgesic effect likewise aspirin and other NSAIDs, of which its effect is suggested to be at the hypothalamic region (Vane, 1987).

The formalin test in mice is sensitive to NSAIDs and other mild analgesic (Hunskaar, *et al.*, 1985). The test consists of two different phases, possibly reflecting different types of pain (Dubuisson and Dennis, 1977, Hunskaar, *et. al.*, 1985). The early phase may be due to direct effect on nociceptors and can be inhibited by centrally acting analgesics such as morphine. In contrast, the late phase may be due to an inflammatory response partly mediated by PGs and can be inhibited by NSAIDs and steroids, as well as the centrally acting drugs. As inflammation occurs at the site of formalin injection it should be possible to elucidate the role of inflammation on the response in the late phases. It was previously shown that aspirin and indomethacin are antinociceptive through partially different modes of action in this test with aspirin having central actions while indomethacin does not. The antinociceptive effect of aspirin in the early phase of the formalin test is due to a central action, which is probably not related to the inhibition of PGs synthesis (Hunskaar and Hole, 1987). It is noted from this study that the methanol fraction was not analgesic active in both early and late phases of the formalin test. On the other hand the hexane fraction showed analgesic activity in both phases of this test. The result showed that the analgesic effect of the hexane fraction was comparable to that of aspirin at the

same dosage range in both phases. The higher percentage of the algesia inhibition of aspirin and hexane fraction in the late phase of the formalin test suggests an effect on acute inflammation. The result on this late phase indicates the inhibitory effect of hexane fraction on the synthesis and/or release of PGs, which is the mechanism of action of aspirin, and NSAIDs. Data from the ear edema test using EPP as an inducer of inflammation confirm the mechanism of action of hexane fraction on cyclooxygenase pathway via inhibition of the cyclooxygenase enzyme in the synthesis of PGs (Hunskaar and Hole, 1986). For the slight inhibition on the early phase, it may be due to direct effects of aspirin and hexane fraction on nociceptor; this phase can be markedly inhibited by centrally acting analgesic like morphine. In this present study, morphine completely inhibited responses in both early and late phases of formalin test.

EPP-induced ear edema formation is a useful model for investigation at an anti-inflammatory activity of a test substance on the acute phase of inflammation. Edema caused by topically applied EPP is due to vasodilatation and increased vascular permeability. This event is caused by the release of various inflammatory mediators such as histamine, 5-HT and PGs (Brattsand, *et al.*, 1982). The methanol fraction did not

exhibit an inhibition of ear edema, but the hexane fraction markedly inhibited ear edema formation induced by EPP. Phenylbutazone, a selective cyclooxygenase inhibitor, could markedly reduce the edema of the ear in this test model. It is suggested that the hexane fraction probably possesses anti-inflammatory activity, likewise phenylbutazone, by inhibition of the cyclooxygenase pathway and other inflammatory mediators of the acute phase of inflammation.

Arachidonic acid-induced ear edema is another skin model of inflammation which is useful for screening compounds showing inhibition on the acute inflammatory reaction (Young, *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 (Young, *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 inhibitors *in vivo*.

Arachidonic acid-induced ear edema probably results from the rapid production (peak concentration by 15 min) of PGE_2 and LTC_4/D_4 (Chang, *et al.*, 1986). These mediators are capable of promoting vasodilatation and increasing vascular permeability and may act synergistically to produce edema. Neutrophils migrate into the dermis of the ear 30 min after arachidonic acid administration and reach high concentration at 1 h when edema is maximal (Chang, *et al.*, 1986). Thus it seems that arachidonic acid metabolites are probably produced by resident skin cell populations and vascular endothelial cells. In contrast to arachidonic acid-induced ear edema, the tetradecanoylphorbol acetate (TPA) or EPP-induced ear edema is primarily mediated by PGE_2 (Carlson, *et al.*, 1986). Humes, *et al.*, (1982) found that TPA or EPP produced a large amount of PGE_2 but only small amounts of 5-lipoxygenase products from the mouse peritoneal macrophages. These biochemical changes occurring with arachidonic acid and TPA/EPP models help to explain the sensitivity of arachidonic acid-induced edema to lipoxygenase inhibitors and why cyclooxygenase inhibitors are especially effective against TPA/EPP model.

The results of the present study showed that phenidone, a nonselective inhibitor of arachidonic acid metabolism, exhibited pronounce edema inhibitory effect on arachidonic

acid-induced ear edema formation whereas phenylbutazone, a selective cyclooxygenase inhibitor, demonstrated no inhibitory activity on this edema model. Methanol fraction did not elicit inhibitory activity on this edema model. Hexane fraction, on the contrary, exerted significant inhibitory effect on ear edema formation induced by arachidonic acid. It is therefore possible that hexane fraction exhibits anti-inflammatory activity in part by inhibition of lipoxygenase pathway.

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; vasodilation, hyperemia, pain, edema and cellular infiltration (Lewis, 1981, Issekutz and Movat, 1982). The cyclooxygenase products, particularly PGE_2, contribute to increased blood flow through a vasodilatation action, but the lipoxygenase pathway is necessary for vascular leakage and edema formation consequent on cellular infiltration (Wedmore and William, 1981)

The hexane fraction possessed pronounced anti-inflammatory activity as seen in EPP-induced ear edema as well as in the late phase of formalin test. These results seem to be

similar to those of aspirin and other NSAIDs. It is likely that the hexane fraction acts via a similar mechanism to elicit the anti-inflammatory activity as NSAIDs by inhibition of the cyclooxygenase pathway. By the way, the mechanism via inhibition of lipoxygenase pathway is also possible, since the haxane fraction showed significant inhibitory effect on arachidonic acid-induced ear edema formation.

Yeast-induced hyperthermia in rats was used to test antipyretic activity of *D. variegata* in this study. This effect is commonly mentioned as one of the characteristics of aspirin and some NSAIDs resulting from their inhibitory effect on the biosynthesis and the release of PGs at the hypothalamus caused by endogenous pyrogens (Flower, 1984; Vane, 1987; Milton and Wendlandt, 1970). The biosynthesis and release of the most potent pyretic agent, PGE_2, appears to be a final step in the common pathway responsible for fever production induced by several pyrogens (Milton, 1982). The results obtained from this study revealed that the methanol fraction did not possess any antipyretic effect whereas the hexane fraction could markedly decrease the body temperature of pyretic animals similarly to aspirin. Therefore, it is suggested that the antipyretic activity of the hexane fraction probably occurs in part in similar fashion

NSAIDs by inhibition of PG-biosynthesis in the central nervous system.

In conclusion, the analgesic, antipyretic and anti-inflammatory activities of stems from the plant *D. variegata* were investigated using many test models. The results revealed analgesic, antipyretic and anti-inflammatory activities of the plant. However, only the hexane fraction, which contains nonpolar substances, possessed moderate analgesic, antipyretic and anti-inflammatory effects. The analgesic activity of the hexane fraction might be mediated by blocking PG-biosynthesis, since PGs, especially PGE_2, are responsible for potentiation of algesia caused by some mediators i.e. bradykinin and histamine. PG production is the final common pathway for causing fever induced by many pyrogens, therefore the hexane fraction probably mediated antipyretic activity on this pathway by inhibition of PG biosynthesis in the central nervous system. The study of the effect of hexane fraction on COX-1 and COX-2 from the laboratory in Uppsala University, Sweden, showed that the hexane fraction inhibited both COX-1 and COX-2 with the percentage of inhibition of 73.04 and 50.2, respectively. Meloxicam, a selective COX-2 inhibitor with lower side effects on gastrointestinal tract and on kidney function, showed the COX-2 : COX-1 inhibition ratio of 0.8 in intact cells (Frelich,

1997). Similarly, the COX-2 : COX-1 inhibition ratio of the hexane fraction was found to be 0.7, which is almost the same ratio as meloxicam. It is therefore possible that the hexane fraction will exhibit a lower rate of gastrointestinal and renal side effects. On the acute inflammation, it is likely that the inhibition of arachidonic acid metabolism and the resulting inhibition of PG and LT biosynthesis are the mechanism of action mediated by the hexane fraction. It is possible that the nonpolar substances present in hexane extract are responsible for the analgesic, antipyretic and anti-inflammatory activities observed in this study. The extract from the stem of this plant has been used in indigenous medicine as a remedy for algesia and inflammation (Panthong, *et al.*, 1986). Thus this present study supports the claimed uses of this plant in folk medicine.