DISCUSSION

In Thai folk medicine *O. integerrima* was used as analgesic, anthelmintic, and antiflatulent agent, also as a refreshing beverage (Pongboonroud, 1971). The plant of the same genus i.e. *O. obsusata* was biologically investigated in India. The results of this investigation showed that the 90% ethanol extract of *O. obsusata* possessed analgesic and anti-inflammatory properties when tested in acetic acid-induced writhing response and carrageenin-induced pedal edema models (Sivaprakasam *et al., 1996*). In this present work *O. integerrima* was selected for the study of its analgesic, anti-inflammatory and antipyretic properties, because no previous research work in biological activities of this plant have been reported.

Ochna extracts were evaluated for the analgesic activities by using the writhing response, the tail-flick and the formalin tests. In anti-inflammatory test, the ear-edema model was used. Yeast-induced hyperthermia in rats was used to evaluate the antipyretic effect. The extract that possessed highest analgesic action was used to assess for toxicity.

The writhing response is used to screen for both peripherally and centrally acting analgesic activity. The abdominal constriction response is thought to involve, in part, local peritoneal receptors (Bentley *et al.*, 1983). This test is widely used for analgesic screening

(Alexandre-Moreira *et al.*, 1999). Acetic acid caused algesia by liberating endogenous substances including H^+, K^+, 5-HT, histamine, PGs, bradykinin, sP and many others that excite pain nerve ending (Raj, 1996). Ochna extracts and aspirin significantly inhibited the writhing response in mice in a dose-dependent manner. Aspirin could decrease the number of writhes by inhibiting enzyme cyclooxgenase, the essential enzyme in the synthesis of PGs, in peripheral tissues. (Fields, 1987). PGs are synthesized at the site of injury and can act upon the peripheral afferent terminal to facilitate afferent transduction and augment the inflammatory state (Yaksh, 1996). The analgesic property of Ochna extracts could probably be due to the blockade of the effect or the synthesis and/or release of endogenous substances that excite pain nerve ending similarly to aspirin.

The tail flick reflex is spinally organized because it persists after midthoracic spinalization. The afferent limb of the tail flick reflex arc consists of thermal nociceptors in the tail, whose unmyelinated afferent fibers travel dorsolateral and ventrolateral tail nerves to terminate in the lateral and medial aspects of laminae I-II of the dorsal horn. The unmyelinated afferent fibers of single intracellularly labeled polymodal nociceptors were shown to terminate in the substantia gelatinosa. The efferent limb consists of motoneuronal cell groups responsible for tail movement (Carstens and Wilson, 1993).

Analgesia can be achieved centrally by interfering with a variety of neurotransmitter systems. The central control of pain is subject to descending modulation by brainstem cell groups such as locus coeruleus/subcoeruleus and raphe complex (Stamford, 1995). These nuclei contain mainly noradrenaline and serotonin, respectively (Bamigbade *et al.*, 1997). Weak analgesic such as aspirin has little or no influence on the response in tests with phasic stumuli, such as the tail-flick and hot-plate tests, while the effects in the formalin test are evident (Tjolsen *et al.*, 1992).

In this test model OI-BK, OI-ST and OI-TW showed some inhibitory effect in tail-flick response but this effect is much lower than that of morphine, while OI-LF and aspirin possessed no inhibitory effect. The result regarding analgesic activity in this study are similar to those reported for morphine and aspirin (Di Stasi, 1988; Zia *et al.*, 1995). It can be proposed that OI-TW, OI-ST and OI-BK exert some antinociceptive effects in this pain model in part by inhibiting the brainstem pathway.

The formalin test in mice is sensitive to NSAIDs drugs and other mild analgesics. The test consists of two distinctive phases, possibly reflecting different types of pain. This method is also very useful for elucidating the mechanism of pain and analgesic effect of substance (Tjolsen *et al.*, 1992). The first phase starts immediately after injection of formalin. It is probably due to direct chemical stimulation of

nociceptors, (Dubuisson and Dennis, 1977, Hunskaar *et al.*, 1985, Tjolsen *et al.*, 1992) and experimental data indicate that formalin predominantly evokes activity in C fibers, and not in Aδ afferents (Tjolsen *et al.*, 1992). The second phase starts approximately 15-20 min after formalin injection and lasts for 20-40 min. The second phase seems to be due to peripheral inflammatory response (Heapy *et al.*, 1987; Hunskaar and Hole, 1987) partly mediated by PGs. Non-steroidal anti-inflammatiory drugs (NSAIDs), such as indomethacin, reduce nociceptive behavior during the second phase, while the first phase seems unaffected (Hunskaar and Hole, 1987). This lack of effect in the early phase persisted even when a very low formalin concentration was used, suggesting that the two phases are qualitatively different and that the difference is not only due to a difference in stimulus intensity (Rosland *et al.*, 1990b). Experimental results have indicated that sP and bradykinin participate in the early phase, while histamine, serotonin, PGs and bradykinin are involved in the late phase (Shibata *et al.*, 1989b).

Morphine and codeine as examples of centrally acting analgesics are antinociceptive in both phases. In contrast, the NSAIDs such as indomethacin and naproxen and the steroids (e.g. dexamethasone and hydrocortisone) inhibit only the late phase, while aspirin was antinociceptive in both phases (Chen *et al.*, Elisabetsky *et al.*, 1995, Hunskaar and Hole; 1987, Santos *et al.*, 1995). In this study it

was shown that the administration of Ochna extracts, morphine and aspirin produced antinociceptive effects on both phases of formalin test, markedly in the late phase. Data obtained from early phase test suggest that analgesic activity of Ochna extracts is mediated via an inhibitory effect on excitation of local nociceptor. The result on the late phase indicates the inhibitory effect of Ochna extracts on the synthesis and /or release of PGs, which is the mechanism of action of NSAIDs and aspirin.

Inflammation is involved in many kinds of pathologic symptoms. Its process is caused by the release of mediators from tissue and migrating cells. PGs, histamine, bradykinins, LTs and many others are the candidates for inflammatory mediators. EPP-induced rat ear edema was used to evaluating inhibitory effect of a test substance on inflammatory mediator release, particularly PGs. All of mediators are capable of promoting vasodilation and increasing vascular permeability as well as synergistically producing edema (Carlson, *et al.,* 1985). Phenylbutazone, a selective cyclooxgenase inhibitor, markedly exhibited an inhibition on ear edema induced by EPP. The result in this study showed that Ochna extracts exhibited a dose-dependent topical anti-inflammatory effect on EPP-induced ear edema in rats. This finding suggests that Ochna extracts probably possess anti-inflammatory activity, likewise phenylbutazone i.e. by inhibiting the cyclooxygenase pathway of the acute phase of inflammation.

Data from EPP-induced ear edema formation and the late phase of formalin test confirmed mechanism of action of Ochna extracts on arachidonic acid metabolism via an inhibition of the cyclooxygenase enzyme in the synthesis of PGs.

Application of AA (0.1-4 mg) to the ears of mice produces immediate vasodilation and erythema, which are maximal at 40-60 min. The onset of edema coincides with extravasations of protein and leukocytes. After 1 h, the edema begins to wane rapidly and the inflammatory cells leave the tissue so that by 6 h the ears have returned to near normal except for residual erythema. Inhibitor studies show that the inflammatory response is due to the formation of AA metabolites in both the cyclooxygenase and lopoxygenase pathways (Young *et al.*, 1984). The response to 12-O-tetradecanoylphorbol acetate (TPA) and EPP can be inhibited or delayed by nonsteroidal agents that act preferentially against the cyclooxygenase pathway of AA metabolism. Inhibition of AA-induced edema appears to be restricted to agents that have the additional property of inhibiting the lipoxygenase pathway of AA metabolism. It appears that products of the cyclooxygenase pathway, particularly PGE_2, contribute to increased blood flow through a vasodilatory action, but that products of the lipoxygenase pathway are necessary for vascular leakage and edema consequent on cellular infiltrate to occur (Young, *et al.*, 1984). Topical application of AA to the mouse ear results in erythema and edema preceded by the

appearance of PGE_2 and LTC_4/D_4 in the tissues. Data of previous studies on topical inhibitors of the cyclooxygenase and the 5-lipoxygenase pathways suggest that the erythema (vasodilation) is due to PGE_2, while the increased vascular permeability causing edema is mainly due to LTC_4/D_4. Phenylbutazone, a cyclooxygenase inhibitor does not produce an inhibition or is inactive, whereas phenidone, a dual inhibitor of AA metabolism shows significantly inhibition in this ear edema model (Young, *et al.*, 1984). The result in the present study showed that all the Ochna extracts could inhibit rat ear edema formation induced by AA. It is therefore possible that Ochna extracts exhibit anti-inflammatory activity in part by inhibition of AA metabolism via the lipoxygenase pathway.

Yeast-induced hyperthermia in rats was used to measure antipyretic activity of *O. integerrima*. One common characteristic of aspirin and other NSAIDs is the inhibitory effect on the biosynthesis and the release of PGs at the hypothalamus caused by endogenous pyrogen (Flower, 1984). Fever may be provoked by many stimuli. Most often, they are bacteria and their endotoxins, virus, yeasts, protozoa *etc*. These substances are commonly called exogenic pyrogens. Cells stimulated by exogenic pyrogens form and produce cytokines called endogenic pyrogens. Endogenic pyrogens centrally affect the thermo sensitive neurons in the preoptic area of the hypothalamus increase the production of heat and decrease in heat loss (Stvrtinova *et al.*, 1995).

The most important endogenic pyrogens are IL-1 and cachectin also called the tumor necrosis factor-α (TNF-α). They are produced especially by monocytes and macrophages but also by endothelial cells and astrocytes (Stvrtinova *et al.*, 1995). In the hypothalamus, IL-1 and TNF-α trigger the synthesis of PGE_2 from the AA of cytoplasmic membranes of target cells. Precise mechanism, by which PGE_2 reset the central thermostat, is not known. Aspirin and the NSAIDs display antipyretic activity by inhibiting the cyclooxygenase, an enzyme responsible for the synthesis of PGE_2 (these antipyretics do not inhibit the production of TNF-α, or IL-1). Glucocorticoids work antipyretically by inhibiting the production of IL-1 and TNF-α, and by inhibiting the metabolic processes of AA (Stvrtinova *et al.*, 1995). The results obtained in the present study showed that all Ochna extracts possessed the antipyretic effect similarly to aspirin. When compared to aspirin, Ochna extracts possessed stronger intensity in lowering rat rectal temperature. Therefore, it can be concluded that the mechanism of analgesic, anti-inflammatory and antipyretic activities of Ochna extracts may occur in part similar to that of aspirin or other NSAIDs i.e. inhibition the biosynthesis of PGE_2. Centrally acting analgesic activity and inhibitory effect on lipoxygenase enzyme are other pharmacological activities of Ochna extracts.

The results of the present study can be summarized that the extracts obtained from leaf, bark, twig, and stem of *O. integerrima* were

proved to possess analgesic, anti-inflammatory and antipyretic activities as claimed in Thai folk medicine. These effects were found markedly in the extract from bark i.e. OI-BK, which indicates that the active constituents contain mostly in bark. Therefore OI-BK was chosen to examine for toxicity. Result obtained suggests that OI-BK possessed high toxicity compared to its effective doses as analgesic and antipyretic activities. As OI-BK is the crude extract which containing numerous different substances, continuing phytochemical work (fractionation and isolation) on this extract using bioassay-guided fractionation may provide more information of constituents possessing therapeutic and/or toxic activity.