DISCUSSION

Inflammation is a localized, protective response to injury and arises from the resultant cell damage (2;4). It involves a complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair (5;7). 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 (3). 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 (90). This fact, associated with the complexity of the inflammatory process, makes the use of different experimental models essential when conducting pharmacological trials. 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 (8). 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 minimal side effects. The search for a safe anti-inflammatory drug with minimal side effects continues unabated and a part of such research is the evaluation of medicinal plants known to be used for the treatment of inflammatory disorders (91). Nowadays, plants have long provided mankind with a main source of new chemical substances with potential therapeutic applicability.

The present study on *G. hanburyi* was carried out because it is used in folk medicine for infected wound, pain and edema. Although no pharmacological effects of *G. hanburyi* have been reported, some species of *Garcinia* are widely used for different types of inflammatroy disease (62;84;85;92).

EPP-induced rat ear edema formation is a useful model for screening the anti-inflammatory activity of test substances on acute phase of inflammation (41). The inflammatory mediators released in this model include histamine, serotonin, bradykinin and PGs, respectively. These mediators are capable of promoting vasodilation and increasing vascular permeability as well as synergistically producing edema (93). The results of the present study showed that the reference anti-inflammatory drug, phenylbutazone, acting by inhibition of COX elicited marked inhibitory effect on edema formation in this model. Similarly, GH5763 could markedly reduce the edema of the ear in this model. It is suggested that GH5763 probably possessed anti-edematous action by inhibition of the COX pathway and/or of other inflammatory mediators of the acute phase of inflammation.

Carrageenin-induced rat hind paw edema is considered to be 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 (43). This suitable test also has frequently been used to assess the anti-edematous effect of natural products (94). The edema induced in the rat hind paw by the local injection of carrageenin is mediated by the initial release of histamine and serotonin (95) followed by the release of bradykinin (95) during the 1^{st} h after carrageenin injection. The second phase of inflammation is due to the release of PGs (46;95). This phase occurs 1.5 - 3 h after carrageenin injection and lasts about 7 h. The release of PGs is closely associated with leukocyte migration to the inflamed area (45). The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at 3 h and periods thereafter (46). It is well established that the second phase is sensitive to most clinically effective anti-inflammatory drugs, particularly NSAIDs (45;46). Oral pretreatment of animals with GH5763 as well as aspirin resulted in a significant inhibition of carrageenin-evoked hindpaw edema. Regarding the possible mechanisms involved in carrageenin-induced paw edema several

inflammatory processes have been suggested to play a role. e.g. activation of complement, and release of histamine, kinins, AA metabolites and pro-inflammatory cytokines (47;96). The significant inhibitory effect of GH5763 on carrageenin-induced paw edema at the 3rd h, suggests that the main mechanism of action of GH5763 may also posses some influence on the other inflammatory mediators e.g. histamine, serotonin, and pro-inflammatory cytokines which are released during the 1st h after carrageenin injection. The results in this test model support the possible mechanism of action of GH5763 on the COX pathway and on other inflammatory mediators, which are involved on paw edema caused by carrageenin. Another possible anti-inflammatory mechanism may involve the other actions of GH5763 on the activation of complement and on the release of pro-inflammatory cytokines.

Recently, it has been shown that LTs, 5-lipoxygenase products of AA, are also involved in inflammatory reactions as proinflammatory mediators. LTC_4 causes edema together with an increased microvascular permeability (97;98) whereas LTB_4 causes leukocyte chemotaxis (99;100). Therefore the inhibitory effect of GH5763 on the lipoxygenase pathway was investigated using the 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 (42;101). 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 (47). Previous studies found that selective COX inhibitors produce nonsignificant inhibition or are inactive in this model, whereas dual inhibitors of AA metabolism show consistently significant inhibition of edema (42;101).

The injection of AA into the hind paw produced significant edema after 1 h. GH5763 at all doses 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. Since GH5763 did not show any activity in this edema, it seems that its anti-edematous action is not related to the lipoxygenase pathway, but it could be related to the inhibition of COX pathway and/or the synthesis/releasing system of other inflammatory mediators, since GH5763 at all doses used in the AA-induced paw edema model, could produce significant inhibition of edema formation in the carrageenin-induced paw edema model.

The difficulty in dealing with 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 serve for investigation of anti-arthritic substances (102;103). The cotton pellet-induced granuloma method 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. 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 (49). Corticosteroids can reduce the transudative weight, via their ability to inhibit the susceptibility of the permeability response occurring around the cotton pellet implantation (104). Most anti-inflammatory drugs (especially corticosteroids) can effectively inhibit the granuloma formation, probably via their ability to interfere with the proliferative component of the inflammatory process (49).

The effect of GH5763, aspirin and prednisolone on the transudative and the proliferative phase of chronic inflammation were determined. The result showed that GH5763 elicited significant inhibitory activity on the transudative and granulomatous weight. In addition, aspirin, a non-steroidal anti-inflammatory drug, elicited significant inhibitory activity on the transudative and granulomatous weight. Prednisolone, a

steroidal drug, exerted marked inhibitory activity on the formation of transudate and granuloma. The result obtained suggest that GH5763 influences the vascular permeability and the proliferative component of the inflammatory process. When assessment was made on the body weight gain and the thymus weight, it was found that GH5763 and aspirin had no effect on the body weight gain and the thymus weight. Prednisolone, on the contrary, markedly reduced the body weight gain and the thymus weight. The loss of body weight gain and the thymus weight in long-term prednisolone treatment may be due to protein catabolism and lymphoid tissue destruction, respectively.(105). 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 suggest a difference in mechanism of anti-inflammatory action of GH5763 and prednisolone since GH5763 did not influence the body and the thymus weight. It is therefore postulated that GH5763 is devoid of steroid like action.

In order to understand the biochemical mode of action of GH5763, 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. Leukocytes accumulate at sites of inflammation and are believed to contribute to tissue damage by releasing lysosomal enzymes and toxic oxygen radical (22). The digestion of ingested material is accomplished by the formation of phargolysosomes through fusion of lysosome with vacuoles surrounding the phagocytosed material and the emptying of lysosome contents into it. Generally, lysosomes are packed with hydrolytic enzymes and cationic protein (7). When leukocytes phagocytize an inflammatory agent, they release lysosomal hydrolase which damages the surrounding tissue and cartilage that can lead to further perpetuation of the inflammation (50;106). 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 steroid drugs via the stabilization of lysosomal membrane (22). Naik and Sheth (107) 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 GH5763 similar to reference drugs, aspirin and prednisolone. These results suggest that one of the possible mode of action of GH5763 may be through the stabilization of the lysosomal membrane.

In the present research, GH5763 was also tested for its ulcerogenic activity. Interestingly, GH5763 was found to have no ulcerogenic effect when compared with the reference anti-inflammatory drugs, aspirin and prednisolone. It is generally accepted that peptic ulcers are caused by a disruption in the balance of aggressive factors and mucosal defensive factors. Aggressive factors are acid and pepsin, *Helicobacter pylori* infection and NSAIDs. Defensive factors are gastric mucus and bicarbonate, gastric mucosal barrier and blood flow and PGs (108;109). NSAIDs, e.g. aspirin, phenylbutazone and indomethacin, are known to induce ulcers during the course of their anti-inflammatory action (110). These drugs induce gastric lesions by inhibiting COX (111). The two isozymes also differ in function in that COX-1 is widely distributed and has "housekeeping" functions, especially gastric cytoprotection. In contrast, COX-2 is an immediate early response gene product in inflammatory and immune cells (112). The toxicity of NSAIDs in the gastrointestinal (GI) system is thought to be related to the lack of selectivity of those drugs with respect to inhibition of COX-1 and COX-2 (113). In the recent years, research on novel drugs to eliminate these side effects has been intensified (8;113), and selective COX-2 inhibitors have become to represent a new pharmacological class of NSAIDs with minimum gastrointestinal toxicity (24;33). Chronic administration of steroids (e.g. prednisolone)

is known to induce gastric ulceration. Steroids influence the inflammatory response by reducing the PGs, LTs, and PAF synthesis that results from activation of phospholipase A_2. (114).The above described experiments demonstrate that the anti-inflammatory activity of GH5763 may be due to the inhibitory of PG synthesis and/or other inflammatory mediators. Hense, the possibility of the anti-inflammatory effect of GH5763 without ulcerogenic effect may be due to PG inhibition by selective action on COX-2, without an important effect on constitutive COX-1. On the otherhand GH5763 is crude extract containing many compounds; some of these may possess cytoprotective effect which could counteract the gastrointestinal effect of the anti-inflammatory compound(s). Whatever the mechanism of action may be, the anti-inflammatory without ulcerogenic effect is a clinically desirable characteristic of novel anti-inflammatory agents.

The writhing response model is widely used for analgesic screening activity of test substances. Writhing response was induced in rat or mice by an intraperitoneal injection of a noxious agent (e.g. acetic acid). The noxious agent such as acetic acid causes algesia by liberating endogenous substance including H^+, K^+, serotonin, histamine, PGs, bradykinin, substance P (sP) and many other that excite pain nerve endings (55;115). 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 (22;23). PGE_2 is also a potent hyperalgesic agent and possesses synergistic effects with histamine and bradykinin which then excite the pain nerve ending in the peritoneal cavity (22). It has long been known that aspirin and other NSAIDs can decrease the number of writhes by inhibiting enzyme COX, the essential enzyme in the synthesis of PGs, in peripheral tissues (116). In the present study, the analgesic effect of GH5763 was determinated by using acetic acid-induced writhing response. GH5763 and aspirin significantly inhibited the writhing response in mice. Data also showed that GH5763 exhibited a dose-related analgesic activity. The results

obtained from many inflammatory models in this study suggest the inhibitory effect of GH5763 on PGs synthesis. The analgesic property of GH5763 can also probably be due to the blockade of the effect or the synthesis and/or release of PGs and/or other endogenous substances that excite pain nerve endings.

The formalin test is different from most models of pain in that it is possible to assess the way an animal responds to moderate, continuous pain generated by injured tissue. Because of this connection to tissue injury, it is believed that the test provides a more valid model for clinical pain than the tests with phasic mechanical or thermal stimuli (117-120).

The formalin test consists of two distinct phases, possibly reflecting different types of pain (57;120-123). The first phase starts immediately after injection of formalin and lasts about 5 min. It is probably due to direct chemical stimulation of nociceptors, (120;121;123) and experimental data indicate that formalin predominantly evokes activity in C fibers, and not in Aδ afferents (123;124). 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 partly mediated by PGs (57;121;124). Experimental results have indicated that substance P and bradykinin participate in the early phase, while histamine, serotonin, PGs and bradykinin are involved in the late phase (125).

The response of the early phase can be inhibited by centrally acting analgesics such as morphine and codeine. In contrast, the late phase which seems to be due to an inflammatory response is partly mediated by PGs and can be inhibited by NSAIDs (e.g. indomethacin and naproxen), corticosteroids (e.g. dexamethasone and hydrocortisone), as well as the centrally acting analgesics (57;126-128). In the present study, it was shown that GH5763 and aspirin produced antinociceptive effects in both phases of the formalin test, but markedly in the late phase. Data obtained from early phase tests suggest that the slight analgesic activity of GH5763 is mediated via an

inhibition on excitation of local nociceptors and/or an inhibition of mediators responsible for pain induction in the central nervous system at the hypothalamic region. The result in the late phase indicates the inhibitory effect of GH5763 on pain arising from inflammation via the synthesis and/or release of inflammatory mediators, especially PGs. The results obtained from the writhing response model and formalin test in this study suggest the analgesic activity of GH5763 probably be due to inhibition if the synthesis abd/or released of PGs and other endogenous substances that cause pain.

The pyrexia (hyperthermia) induced in rat by brewer's yeast (subcutaneous injection) has been used to determine antipyretic activity of many compounds (59). The antipyretic activity of GH5763 was investigated in yeast-induced hyperthermia in rats. The regulation of body temperature requires a delicate balance between the production and loss of heat; the hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point is elevated. Fever may be provoked by many stimuli, such as bacteria and their endotoxins, viruses, yeast, spirochets, protozoa, immune reactions, several hormones, mediations and synthetic polynucleotides. These are commonly called exogenic pyrogens. Cells stimulated by exogenic pyrogens form and produce cytokines called endogenic pyrogens. Endogenic pyrogens centrally affect the thermosensitive neurons in the preoptic area of the hypothalamus and increase the production of heat and decrease in heat loss. The body temperature increases until it reaches the new set point (1;129-131).

The most important endogenic pyrogens are IL-1 and cachetin, also called the tumor necrosis factor-α (TNF-α). They are produced especially by monocytes and macrophages but also by endothelial cells and astrocytes. Also the interferons (IFN) α, β and γ display pyrogenic activity (129;131).

After administration of an endotoxin in an experiment, the level of plasmatic TNF-α increases and fever occurs. Increased concentrations of IL-1 and TNF-α are

also found in sepsis. The production of these cytokines is regulated by the positive feedback mechanism. Beside this, macrophages activated by TNF-α may increase the production of IL-1 and TNF-α initially induced by other stimuli in the hypothalamus, IL-1 and TNF-α trigger the synthesis of PGE_2 from the AA of cytoplasmic membranes of target cells. The precise mechanism by which PGE_2 resets the central thermostat is not known. Aspirin and the NSAIDs display antipyretic activity by inhibiting PGs synthesis in CNS.(129;131-133).

GH5763 and aspirin showed antipyretic activity causing a decrease in body temperature of hyperthermic rats induced by brewer's yeast. It is possible that the antipyretic activity of GH5763 is due to the inhibition of the synthesis and release of PGs in CNS and/or inhibition of endogenous pyrogens.

Overall, on the basis of the results obtained it suggested that GH5763 shows anti-inflammatory, analgesic and antipyretic activity. The anti-inflammatory effect of GH5763 was found prominently on the acute phase of inflammation. The effective anti-inflammatory effect of GH5763 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 is likely that GH5763 reduces inflammation by inhibiting the cyclooxygenase pathways of arachidonic acid metabolism and other mediators e.g. histamine, serotonin, kinins etc. In the chronic inflammatory, GH5763 inhibits the transudative phase and fibroblast proliferation. Moreover, GH5763 appeared to be devoid of steroidal like effects such as causing marked decrease of the normal body weight gain and the thymus weight. GH5763 reduced alkaline phosphatase activity, in serum, which might be due to its stabilization activity on lysosomal membrane of leukocyte. The administration of GH5763 did not cause gastric mucosal lesions when compared with aspirin. The anti-inflammatory action of GH5763 can be postulated to have a selective inhibitory effect on COX-2 rather than COX-1. The analgesic and antipyretic activity of GH5763 might be mediated by

inhibition of PG-biosynthesis and other mediators. Further study of pharmacological effects should be conducted to find out the active principles to be potential candidates as novel safe therapeutic agents that are free from gastric intolerance for the treatment of inflammatory diseases. Toxicity study should also be included.