DISCUSSION
Our laboratory has been actively engaged in evaluation of Indian medicinal plants used in traditional medicinal system, in modern scientific terms since the last few years. In course of such investigations the evaluation of pharmacological actions of the methanolic fraction of *M. cordata* (Burm) root with special reference to anti-inflammatory effect has been attempted by the present worker.

Inflammation has been the subject of recent reviews, symposia and books. The discovery of new drugs in this area of research is taking very rapid strides. The complexity of the inflammatory process and the diversity of the drugs that have been found to be effective in modifying this process, has resulted in the development of numerous methods of assay capable of detecting anti-inflammatory substances. A few of these methods have achieved popularity because of their simplicity, reproducibility, economic feasibility and ability to select drugs known to afford some benefit in clinical management of inflammatory diseases.

According to Swingle (1974) the anti-inflammatory screening procedure is composed of the following steps.

1) An interference with the manifestation of one of the cardinal signs of inflammation.
2) Modification of one of the events accompanying the inflammatory process.
3) Modification of those syndromes in laboratory animals which are believed to represent models for various rheumatoid states.

In course of screening indigenous medicinal plants for biological activity in our laboratory it was initially observed that the methanolic fraction of M. cordata root extract significantly inhibited the carrageenin induced paw oedema in rats (Table - 1) and produced effects similar to other 'true' anti-inflammatory agents; these facts prompted us to carry out a detailed evaluation of the anti-inflammatory property of the M. cordata root extract. The present studies were conducted with the methanolic fraction of the extract of M. cordata roots and later on an attempt was made to isolate and identify (at least qualitatively) the nature of the active principle(s) responsible for anti-inflammatory action.

The effect of the M. cordata root extract was examined on the manifestation of cardinal signs of inflammation. For screening and evaluation of anti-inflammatory agents, one of the most commonly employed techniques is based on the ability of the agent to inhibit oedema produced in the hind paw of rats by injection of phlogistic agent. In this context several oedemogens have been proposed to be used by various workers e.g. kaolin,
formalin, yeast, albumin, dextran. However certain observations discussed below limit the use of some of the phlogistic agents in producing experimental inflammation in animals.

It has been reported by Winter et al. (1962) that oedemogenic activity of kaolin diminishes when the substance is suspended in sterile media; dextran-induced oedema are inhibited by chlorpromazine, antihistaminics and by some adrenergic agents, but not by cortisone and phenylbutazone and as well seems to be ineffective unless given at a toxic dose (Winter et al. 1962). Formalin-induced oedema does not appear to be as sensitive to inhibition by phenylbutazone and other related drugs, as are oedemas induced by other irritants, moreover indomethacin and flufenamic acid are ineffective in this test at doses known to produce significant inhibition by other assays. Anti-histaminic agents are also effective in this method (Winter, 1965b). Yeast-induced oedema is relatively insensitive to standard anti-inflammatory drugs (Winter, 1963b). In dextran and ovalbumin-induced oedema also, phenylbutazone seems to be ineffective unless given at toxic doses. Oedema induced by dextran also could be influenced nonspecifically (Winter 1966a; Garrattini et al., 1965). On the contrary, carrageenin-induced oedema in rat paw is used extensively for its reproducibility and specificity.
to anti-inflammatory drugs and being least affected by non-specific factors like ambient temperature, humidity, drug-induced hypothermia, diuresis, hypotension (Garattini et al., 1965) and variations in strain, sex or body weight (Swingle, 1974). Standard anti-inflammatory agents are also effective in this model and yield linear and parallel dose response curves (Winter, 1965b; Swingle et al., 1971d). Accordingly the effect of *M. cordata* root extract on the carrageenin-induced oedema was examined. The root extract in doses 25, 50 and 100 mg/kg (i.p.) significantly inhibited the carrageenin-induced oedema. ED$_{50}$ of the root extract in this model was calculated out to be 48.41 mg/kg (i.p.); it may be mentioned here that the root extract did not produce any mortality up to a dose of 1500 mg/kg (i.p.). The ED$_{50}$ of the root extract is higher than that of phenylbutazone and ibuprofen which was found to be 42.45 mg/kg and 30.54 mg/kg respectively, while it was found to be much lower than *Boswellia serrata* (SALLAKI), a herbal anti-inflammatory drug (ED$_{50}$ 158.48 mg/kg, i.p.) introduced in the Indian market. Thus from such observations it may be stated that *M. cordata* root extract effectively inhibited the oedema which is one of the cardinal signs of inflammation.

The development of oedema in rat paw following the injection of carrageenin is a one as biphasic reported...
by Vineger et al., 1969, who further demonstrated that the second phase of the oedema is sensitive to inhibition by drugs like hydrocortisone, phenylbutazone and indomethacin. However, Winter et al., 1962 and Bolam et al., 1974 failed to notice such biphasic responses. These discrepancies might be due to the fact that the early phase is very transient in nature and the same can be detected only on measurements of foot volume in the first hour after carrageenin administration. Doherty and Robinson, 1975 reported the early phase to be due to the trauma of injection. Van Arman et al., 1965 also put forward a similar explanation.

From the experiments concerning involvement of mediators in the carrageenin-induced paw oedema it was suggested that there are three phases of oedema, i.e., the early phase attributed to the release of histamine and serotonin, an intermediate phase mediated by kinin-like substances and the next phase due to the release of prostaglandin like substances (DiRosa and Willoughby, 1971; DiRosa et al., 1971a). The recognition of various mediators for different phases of oedema has important implications towards interpretation of the effects of drugs. Cyproheptadine, a histamine and serotonin antagonist, has been reported to be ineffective in these assays (Winter, 1965; Vineger et al., 1969). However, pre-treatment of rats with the amine depletor compound...
48/80, abolishes the early phase of the oedema and pre-treatment with antihistaminic substances in combination with cyproheptadine partially antagonize the early oedematous response (Di-Rosa et al., 1971a). In rats, kininogen depletion substantially reduces the intensity of the intermediate phase of oedema while the administration of antiproteases suppresses it altogether (DiRosa and Sorrentino, 1968; Van Arman et al., 1965). Proteolytic release of kinins may account for the first phase of oedema as suggested by Vinegar et al., 1969. The involvement of prostaglandin like substances in the third phase of oedema demonstrated by Willis (1969) confirmed the findings of DiRosa and Willoughby (1971).

We have demonstrated in our present experiments that the increase in paw volume consisted of an early transient phase reaching its maximum at 60 minutes and a late phase which had its maximum effect of 3 hours after carrageenin administration (Table 2 and Fig.9 in Results). Thus our findings are in conformity with the observations of Vinegar et al., 1969 who also noted a biphasic response and a maximum increase at a similar point of time. Root extract of M.cordata at a dose level of 100mg/kg, i.p., reduced both the early and late phases of carrageenin-induced paw oedema (Table 2 Fig.9 in Results Chapter). Although Vinegar et al., 1969 noted that only the second phase of oedema is susceptible to inhibition.
by standard anti-inflammatory drugs (e.g. Phenylbutazone, Hydrocortisone, Indomethacin) but *M. cordata* root extract inhibited both the phases. However, in the experiments performed by us phenylbutazone effectively antagonised both the phases of oedema and this is in contradiction to that reported by Vinegar et al., 1969.

We have already discussed that the early phase of carrageenin oedema was attributed to the release of histamine. It may be mentioned in this context that the root extract could not be demonstrated to possess any antihistaminic action as the extract failed to antagonize histamine induced hypotension in cats while root extract significantly inhibited the histamine and serotonin-induced paw oedema in rats. The later phase of oedema is attributed to release of prostaglandin like substances. The root extract was found to cause significant inhibition of PGE₁-induced oedema. Although the intermediate phase of carrageenin-induced paw oedema has attributed been release of kinin like substances, in the current experiments the root extract could not be found to significantly antagonize the bradykinin-induced paw oedema in rat.

Effect of *M. cordata* root extract on carrageenin-induced oedema in rats seems to be mediated (at least partially) through pituitary-adrenal axis. This was evident from the observation concerning the comparison of the anti-
inflammatory effect of M. cordata root extract on carrageenin-induced oedema in adrenalectomized and non-adrenalectomized animals it was observed that M. cordata root extract at a dose of 100 mg/kg, i.p. restricted the mean oedema volume (±SE) to 0.153 ± 0.02 ml in non-
adrenalectomized rat while in adrenalectomized rats the mean oedema volume (±SE) was 0.29 ± 0.03 (Table 3 and Fig.10 in Results) the difference being statistically significant. Accordingly the anti-inflammatory activity of M. cordata seems to be dependent on the pituitary-adrenal axis at least partially. It is worth to mention here that number of non-steroidal anti-inflammatory drugs were reported to be partially dependent on pituitary-adrenal axis (Parmer and Ghose, 1978).

The carrageenin-induced oedema is subject to 'non specific' inhibition by irritants such as hypertonic saline, dilute acetic acid, formalin, croton oil, carrageenin, kaolin and anti-inflammatory exudates (Zarrattini et al.,1965; Waiz et al.,1970; Atkinson and Hickey,1971) or toxic agents like carbon tetrachloride and mercuric chloride (Medina et al.,1969). Shanahan (1968) has reported that 'anti-inflammatory' substances that exert their effect by virtue of their irritant properties can be distinguished from 'true' anti-inflammatory agents by administering them locally with carrageenin in a mixture and such administration of irritant compounds produce
a further increase in the size of the paw, whereas non-irritant anti-inflammatory agents produce a reduction in the size of the paw. In our experiments when M. cordata root extract was administered in a mixture with carrageenin into rat paw, it was observed that the carrageenin-induced oedema was significantly reduced (Table 4 in Results). This observation suggests that the inhibition of the carrageenin-induced paw oedema by the root extract is due to its 'true' anti-inflammatory activity rather than any irritant property.

It is also desirable that an anti-inflammatory drug should be effective orally otherwise it will lose much of its credibility. Accordingly the effect of oral administration of M. cordata root extract was examined on carrageenin-induced oedema and it was found that the root extract at the dose of 100 mg/kg on oral administration, caused a 59.36% inhibition of carrageenin-induced oedema, whereas on administration of the same dose through intraperitoneal route caused a 67.17% inhibition. From the above findings it is evident that the root extract of M. cordata is also effective in its anti-inflammatory activity through oral route although a little lesser in magnitude.

We have discussed in detail regarding the mediators of inflammation earlier (Review of Literature). Histamine, serotonin, kinin and prostaglandin are known to
mediate the production of various phases of carrageenin-induced paw oedema (DiRosa et al., 1971a). Prostaglandin is a mediator in the last phase of the inflammatory response of carrageenin-induced paw oedema as demonstrated by Willis (1969). Hyaluronidase is known to act by polymerizing the hyaluronic acid of capillary endothelium and there by producing an increase in the vascular permeability (Ghosh et al., 1965). Different mediators of inflammation have been employed as oedemogens in a rational approach towards evaluation of newer anti-inflammatory drugs.

Each of M. cordata root extract was examined on oedema induced by histamine, serotonin, hyaluronidase, prostaglandin (PGE₂) and bradykinin. There was significant inhibition of paw oedema induced by all of the mediators excepting bradykinin. The root extract was administered at a dose of 100 mg/kg in each type of mediators(s)-induced oedema. So from the above observations it may be concluded that the root extract inhibits the oedema induced by different inflammatory mediator(s) e.g. Histamine, serotonin, Hyaluronidase and Prostaglandin (PGE₂) but not bradykinin at least in the dose employed (i.e. 100 mg/kg). It may be recalled here that though the root extract antagonized histamine or serotonin induced-oedema in rat paw, no antihistaminic or anti-serotonergic activity, could be observed to occur
with the extract since the extract failed to antagonize histamine or serotonin-induced fall of blood pressure in cats.

The effect of the *M. cordata* root extract was examined on turpentine-induced joint oedema at different time intervals up to 5 hours. There are same sequential release of the mediators in turpentine-induced oedema as released during carrageenin-induced oedema e.g. histamine and serotonin in early phase, kinin like substance in intermediate phase and prostaglandin in late phase (DiRosa et al., 1971a).

The root extract produced significant inhibition of turpentine-induced joint oedema in rats at 1st, 4th and 5th hour after injection of turpentine (Table 7, Figure 14 in Results). Hanson et al., 1974 reported that inflammatory response is biphasic in this test (turpentine-induced joint oedema) and that a period of 4 hours includes both phases of the response. In our experiments we have examined the effect of *M. cordata* root extract up to 5 hours. In the 1st hour after turpentine injection there was significant reduction of inflammatory response, the occurrence of which is attributed to histamine and serotonin but in the 2nd hour and 3rd hour after turpentine injection there are no significant reduction of inflammatory response which is attributed to kinin like compounds and it may be once again men-
tioned here that the root extract was found to be ineffec-
tive against kinin-induced oedema (Table 6). While
in the 4th hour the root extract significantly inhibited
the inflammatory response and the phase of oedema is
attributed to be due to involvement of prostaglandin-
like substances. There were also significant reduction
of the inflammatory response at 5th hour. Here in our
experiment phenylbutazone was found significantly effec-
tive against the response throughout the 5 hours. These
results demonstrate that the *M. cordata* root extract
significantly inhibited the inflammatory response indu-
ced by turpentine up to the end period of observation
(i.e. 5th hr.).

Antipyretic and anti-inflammatory activity are not inse-
parable, although these two properties occur altogether
in acidic non-steroidal anti-inflammatory agents with
surprising regularity. The mode of anti-pyretic action
of non-steroidal anti-inflammatory drugs is not yet
fully clear. It has been suggested that PGH mediates
pyrogen fever, the ability of non-steroidal anti-inflam-
matory drugs to inhibit prostaglandin synthesis can
help to explain their antipyretic activity (Feldberg
and Saxena, 1971) but of course it is not the sole rea-
son.

We utilized yeast-induced pyrexia in rats to evaluate
the antipyretic action of *M. cordata* root extract; it
was noted that the root extract had a definite anti-pyretic property. At the doses of 50 and 100 mg/kg (i.p.) the root extract considerably reduced the febrile response in rats. An appreciable reduction of temperature had been noticed within the 1st hour after administration of *M. cordata* root extract (Table 8 Figure 15 in Results). At the 2nd hour after the administration of root extract the inhibition was maximum (Table 8 Fig. 15 in Result chapter) and the effect of root extract (100mg/kg) was almost comparable with aspirin (100mg/kg) at this point of time.

So we may conclude that the *M. cordata* root extract (50 and 100 mg/kg) possess significant antipyretic activity although aspirin (100 mg/kg) being more potent than the root extract throughout the experiment except only in 2nd hour while both were equally comparable.

Pain is one of the cardinal signs of inflammation. Hence, it is necessary to evaluate whether any new drug modify the inflammatory pain which appears to be the most relevant test because this type of pain is present in most of the conditions for which the anti-inflammatory drugs are used. For this purpose acetic acid-induced writhing response in mouse was used to examine the effect of *M. cordata* root extract, since the method is not only simple and reliable but also affords rapid evaluation of this type of algesic responses. It was found that
M. cordata in the doses employed significantly inhibited the acetic-acid-induced writhing response in mice (Table 9 Fig.16 in Results chapter). It effectively reduced the wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limb occurred in mice due to the nociceptive property of acetic acid.

Next in order to distinguish between central and peripheral analgesic activity of root extract of M. cordata tail clip method in mouse was used. It is known that the centrally acting analgesic drugs elevate the pressure threshold of mouse following the application of tail clip. It was noticed that M. cordata failed to raise the pain threshold which indicated that the analgesic property of the root extract is not centrally mediated, as narcotic analgesics effectively raise the pain threshold in tail clip experiment (Table 34 in Result Chapter). It has been reported that besides nonsteroidal anti-inflammatory agents, antihistamines, anticholinergics and sympathomimetics can inhibit the writhing responses (Swingle 1974). The possibility of M. cordata having an antihistamic and anticholinergic or sympathomimetic actions are excluded by our experimental studies as on earlier occasions it has been observed that the root extract could not be observed to antagonize the acetylcholine-induced contraction.
of guineapig ileum or histamine or acetylcholine induced fall of blood pressure in Cat. M. cordata also did not influence the adrenaline-induced rise in blood pressure. Thus it may be concluded that the inhibition of algesia as observed by reduction of writhing response by M. cordata root extract is almost definitely due to its anti-inflammatory effect.

Attempts were made to examine the effects of M. cordata root extract on several phases of events during inflammatory process i.e. exudation of fluid (protein exudation and protein-bound dye leakage), migration of leucocytes and formation of granulation tissue.

The inflammatory exudate has two components: the fluid and cells. An important characteristic of the inflammatory reaction is a sustained elevation in permeability of small blood vessels, to protein, which occurs sometime after an initial transient increase in permeability. The initial phase involved primarily the venules, whereas the delayed phase of increased permeability involves the capillaries as well. In general the immediate phase can be inhibited by antagonists of known endogenous permeability factors. The delayed phase of increased permeability of small vessels which is more relevant to chronic inflammation, is generally refractory to such antagonists. Increased vascular permeability and oedema formation are separate phenomenon and this is
not necessarily inconsistent. Oedema without increased permeability can occur from a simple elevation of capillary pressure. The occurrence of increased permeability without formation of oedema should be possible if circulatory and lymphatic sufficiency is maintained in the inflamed area (Swingle, 1974). For assessing the anti-inflammatory activity of test substances, antagonism of the increased vascular permeability occurring in a variety of experimental inflammations has been the criterion of activity. Artificial peritoneal or pleural inflammation induced in mice or rats have been utilized to test the anti-inflammatory effect of drugs. It has already been mentioned that as the vascular permeability increases early in the inflammatory process, the amount of dye-labelled plasma protein exuding into the peritoneal fluid, has been exploited to estimate intensity of inflammation (Northover, 1963; Whittle, 1964). In our experiments the influence of root extract of M. cordata was examined on the intensity of peritoneal inflammation by direct measurement of exuded plasma protein using Biuret reagent after Tomisawa and Sato (1973) and leakage of the protein-bound dye into the peritoneal cavity after Filderman and Kovacs (1969). The increased protein in the peritoneal fluid of mouse after
acetic acid injection appears to be derived from plasma.

The root extract (at a dose of 100 mg/kg) prevented the rise in protein concentration in the peritoneal fluid. This inhibition was slightly greater than aspirin (100 mg/kg) used as standard drug in our experiment (Table 10 in 'Result').

In the protein-bound dye leakage in mouse peritoneal capillary permeability test the steroidal anti-inflammatory agents have been mentioned to be ineffective while non steroidal anti-inflammatory drugs are generally active in this test (Northover, 1963, 1964). We have utilized peritoneal capillary permeability test after Filderman and Kovacs (1969). It was observed that the root extract of M. cordata (when administered in a dose of 100 mg/kg intraperitoneally) demonstrated a significant inhibition of dye leakage. Aspirin (300 mg/kg, i.p.) was also employed in this test as a standard drug which was found to be more potent than root extract in the dose employed. So according to the results of the test, the root extract seems to act in a fashion similar to the non-steroidal anti-inflammatory drugs. From the above observations it may be concluded that the root extract inhibits enhancement of the vascular and capillary permeability both of which being characteristics of the inflamma-
The migration of leucocytes into the inflamed site is another major event that occurs during inflammatory reaction (Review of Literature). Practically, any inflamed area may be used to determine the degree of cellular exudation.

We utilized the carrageenin-induced pleurisy in rats for examining the effect of M. cordata root extract on the migration of leucocytes. The root extract significantly inhibited the migration of leucocytes in the pleural exudate (Table 12, Results Chapter). Phenylbutazone (100 mg/kg) was employed as standard drug in this model which was found to be more potent than root extract in this test.

The repair phase of inflammation is characterized by the proliferation of fibroblasts and multiplication of small blood vessels which typically occur after the vascular and exudative changes. The proliferated cells penetrate the exudates, producing a highly vascularized reddish mass termed 'granulation tissue'. An ideal anti-inflammatory agent should inhibit the formation of granulation tissue. There are number of methods towards testing their effects on the formation of granulomas. However, cotton pellet-induced granuloma and carrageenin-induced granuloma pouch
tests have been employed extensively and the effects of *M. cordata* root extract were tested on formation of granuloma by both these models. In the cotton pellet induced granuloma test, the root extract significantly reduced the weight of granuloma, although phenylbutazone, (100 mg/kg) was more effective than the root extract in this test (Table 13 in Results, page 180). Size of the pellet (i.e. surface area) may influence the formation of increased amount of granulation tissue (Robinson and Robson, 1964) and this variability of response was avoided by controlling the size of the pellet. The pellets which were implanted were of uniform diameter and size. It has been suggested, any test substance when administered by parenteral route, there is a possibility of non-specific inhibition of the granuloma formation particularly when the substance is an irritant one (Cygielman and Robson, 1963). In our case the root extract was administered parenterally in this test since it has already been proved that the root extract of *M. cordata* inhibited carrageenin-induced paw oedema due to its true anti-inflammatory activity and not by its irritant property as it did not increase but decrease the paw oedema when the same was administered simultaneously with carrageenin in rat paw (Table 4, in Result chapter) as suggested by Shanahan (1968). Accordingly, it may
be inferred that the inhibitory effect of root extract of \textit{M. cordata} on granuloma formation was neither due to non-uniform size nor due to non-specific irritant effect but rather definitely due to anti-inflammatory effect.

The effect of root extract of \textit{M. cordata} on granuloma formation was further confirmed on a second model. In the carrageenin-induced granuloma pouch model, \textit{M. cordata} (S.C.) effectively reduced the weight of the pouch wall. Standard drugs employed in this test were phenylbutazone (100 mg/kg/day, S.C.) and indomethacin (5 mg/kg/day, S.C.). The root extract was found to be more potent than phenylbutazone (100 mg/kg/day, S.C.) while Indomethacin (5 mg/kg/day, S.C.) was found more potent than the root extract in this test.

Accordingly it may be stated that the \textit{M. cordata} root extract significantly inhibits the granuloma formation which an important event in the process of inflammation.

Experimental arthritis can be induced in animals by a variety of tests although their similarity to human disease is a matter of considerable debate. However, \textit{M. cordata} was tested for its efficacy in the following models of arthritis in rats.
In the formaldehyde-induced arthritis, the *M. cordata* root extract (50 and 100 mg/kg) significantly reduced the diameter of the inflamed paw (Table 15 in Result chapter). On administration of the root extract daily for 10 days, there was a marked improvement in the arthritic condition in rats. Phenylbutazone (100 mg/kg) employed as a standard drug in this experiment was found to be slightly more potent than the extract at the end of the experiments i.e. on the 10th day.

The observation of arthritic response pattern induced by formaldehyde has also been studied daily (Fig. 22b in Result chapter). It was interesting to note that in animals treated with *M. cordata* root extract (100mg/kg) inhibitory effect were found to be more than that of animals treated with phenylbutazone (100 mg/kg) upto 7th day. From 8th day and onwards phenylbutazone (100 mg/kg) was more inhibitory to arthritis than that of root extract (100 mg/kg/day). It was also observed that root extract (100 mg/kg/day), produced significant inhibitory effect from 2nd to 10th day whereas phenylbutazone (100 mg/kg/day produced significant effect only from 4th to 10th day in this model.)
The root extract in a dose of 50 mg/kg/day demonstrated significant effect only on the 9th and 10th day. So from the overall observations of the results obtained in this test it may be concluded that the root extract (50 and 100 mg/day/day) significantly inhibited the formaldehyde-induced arthritis in the inflamed paw and the effect of the root extract at the dose of 100 mg/kg/day was almost comparable with phenylbutazone (100 mg/kg/day) in respect to their anti-arthritic actions upto 7th day in this model although phenylbutazone (100 mg/kg/day) was found to be more potent in this respect during the period of 8-10 days.

Freund's complete adjuvant-induced arthritis in rats is probably the best and the most widely used model which has a close similarity to human rheumatoid disease. Effect of M. cordata root extract was examined on the inflammatory responses of the disease in rats by the adjuvants-induced arthritis. For assessing the effect of the root extract on this model it was utilised through regular protocol i.e. by administration of root extract on day -1,0, through day 13 (i.e. total 14 days).

On administration of adjuvant into one hind paw of the rat, a pronounced swelling appears shortly afterwards in the injected paw which persists for weeks.
This is usually considered as a primary reaction. After a few days the contralateral paw as well as the front paws also become swollen and arthritic nodules appear on the ears and tail. This is the delayed systemic response. The reaction that occurs at the injected paw does not have the same significance as the disseminated arthritis.

For evaluation of the anti-inflammatory property of *M. cordata* the magnitude of swelling of the hind paws (in both adjuvant injected and non-injected paws) were taken into consideration. The oedema of the injected (adjuvant) paw was used for evaluating the inflammatory response following adjuvant injection while the increase in the size of the non-injected paw was measured to estimate the delayed immunologically-mediated component of the disease.

Root extract of *M. cordata* in doses of 25, 50 and 100 mg/kg/day (when administered following the regular protocol) was found to cause significant inhibition of the paw oedema in the injected paw in a dose dependent fashion. Inhibitory effect at 25 and 50 mg/kg/day of the root extract exerted appear to be almost similar in magnitude (Table 16 in Results). Root extract at a dose (100 mg/kg/day) significantly inhibited the inflammation in the non-injected paw, aspirin (100mg/
kg/day) significantly inhibited the oedema in both injected and non-injected paws and was found to be more potent than the root extract. So from the findings of the test it may be inferred that the root extract has a significant effect in preventing the primary systemic response (at the doses employed i.e. 25, 50 and 100 mg/kg/day) and as well significantly effective against delayed systemic response (in a dose of 100 mg/kg/day).

The degree of severity of the lesions of paws, ears and tail were also assessed subjectively. The root extract inhibited the degree of arthritic lesions to an appreciable extent (Table 16(b) in Results). Thus it may be mentioned that M. cordata root extract is capable of inhibiting the developmental phase of arthritic disease as evident from the results in this model.

Effect of M. cordata root extract was also examined on experimental model of gout. The experimental method is based upon the knowledge that microcrystalline sodium urate is responsible for initiating the attack of gout. The root extract (100 mg/kg) was found to cause significant inhibition of the monosodium urate-induced gout, thereby suggesting the drug to be useful in gouty conditions (Table 17 Figure 24 in Results).
Thus from the results of series the investigations on experimental inflammation in animals, it is evident that *M. cordata* root extract effectively inhibited the cardinal signs of inflammation e.g. swelling, pain, temperature and also the accompanying parameters of the inflammatory process i.e. inhibition of the exudation of fluid, increase in capillary permeability (as evident from inhibition of dye-leakage), migration of leukocytes, formation of granulation tissues and the extract has been found effective against experimental arthritis and gout. All these observations suggest that *M. cordata* root extract may be an useful anti-inflammatory agent.

In order to correlate the anti-inflammatory activity with biochemical changes, the effect of *M. cordata* root extract on transaminases and adenosine triphosphatase activity, was studied in normal and arthritic rats. It has been proposed that the anti-inflammatory drugs have effect on certain enzyme system like transaminases (Steegle et al., 1961; Suggs et al., 1961). On examination it was observed in our experiments that the levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) increased in the sera of formaldehyde - induced arthritic rats (Table 18(a)(b) and Figure 25,26 in Result Chapter).
M. cordata in a dose of 100 mg/kg, i.p., prevented the rise in the enzymatic activity which were associated with the inflammatory reaction. The SGOT activity remained unaltered with M. cordata root extract treatment but significant alteration of the SGPT activity in normal (non-arthritic) rats was observed to occur. The inhibition of transaminase activities by the root extract may be related to its anti-inflammatory action or may be an inherent effect on these enzymes. Since the root extract did not affect the SGOT activity in normal animals but significantly reduced the enzyme activity in inflammatory condition, it may be concluded that the effect of M. cordata root extract on SGOT activity is related to its anti-inflammatory effect. However, such relationship could not be established with SGPT activity, since the root extract showed an inhibitory effect on this enzyme also in normal non-arthritic animals. In a study of acute inflammation due to viral hepatitis, the serum transaminase activity was found to be increased and prednisone reduced it (Picchio and Benda, 1958). Huggins et al., 1961 reported that the inhibition of normal glutamic pyruvic transaminase was greater than glutamic oxaloacetic transaminase in vitro studies with salicylates. Steegle et al., 1961 have reported the inhibitory activity of Salicylate congeners on the SGPT in relation to their...
chemical structure. However, Manso et al., 1954 failed to observe the inhibitory effect of salicylates on the serum transaminases. Results of our studies are also similar with the earlier findings of specific inhibition of the SGOT activity by other anti-inflammatory agents (Kalyanpur et al., 1968; Yangri et al., 1965; Gupta et al., 1971; Naik and Seth, 1978). The inhibition of transaminases that has been observed to occur with anti-inflammatory drugs could influence the continuous formation of polypeptide kinins during an anti-inflammatory process (Whitehouse, 1964). The inhibitory effect may also be responsible for decreased synthesis of mucopolysaccharides, which are mainly concerned with the proliferative phase of inflammation (Gupta et al., 1971).

It has been claimed that salicylates exert their anti-inflammatory effect by uncoupling the oxidative phosphorylation (Whitehouse, 1964). Falcone, 1959 also noted that salicylates uncouple the oxidative phosphorylation by stimulating adenosine triphosphatase (ATPase) activity. In our study concerning the effect of root extract of M. cordata on liver ATPase it was found that ATPase activity in the liver homogenates remained unaltered by the inflammatory reaction (Formaldehyde-induced arthritis). But M. cordata significantly raised the enzymatic activity in the liver homogenates of
both normal (non-arthritic) and formaldehyde-induced arthritic rats (Table 19, in Results).

As the inflammatory reaction did not alter the level of ATPase activity it may be concluded that regarding the mode of anti-inflammatory action of root extract of *M. cordata*, ATPase is not involved and perhaps this is an inherent property of the drug. Similar stimulation of ATPase has been observed to occur also with salicylates, phenylbutazone, corticoids, indomethacin and other anti-inflammatory drugs (Whitehouse and Haslam, 1962; Kalyanpur et al., 1968, Gupta et al., 1971). Accordingly the effects of *M. cordata* root extract on ATPase activity are in conformity with the observation with other standard anti-inflammatory drugs in this context.

The changes in the connective tissue metabolism are some of the major biochemical events during the inflammatory process. These changes lead to variations in the relative composition of different constituents of the connective tissue. Mammalian connective tissue is essentially composed of three elements: specialized connective tissue cells, structured fibres and amorphous ground substance. Many biochemical and pharmacological studies of the activity of anti-inflammatory drugs have been primarily aimed at describing the drug-induced changes in the fundamental components of the ground
substances particularly the acid mucopolysaccharides, (also known as glycosaminoglycans) and the structural protein of collagen fibres. Collagen is the structural protein of collagen fibres which are composed of rod-like tropocollagen molecules. Tropocollagen molecules are built up to form three polypeptides. The tropocollagen molecules are synthesized within the fibroblasts. Collagen biosynthesis follows the basic pattern of biosynthesis but with a few rather unique features. The greatest important feature is the presence of hydroxyproline and hydroxylysine (amino acids found almost exclusively in collagen in the tissues of vertebrates). They are not directly incorporated into collagen but are formed within the macromolecule from proline and lysine. These two amino acids after activation are incorporated into a high molecular peptidic precursor of collagen (procollagen) which is a substrate for the subsequent hydroxylation mediated by the enzyme procollagen proline hydroxylase. In the final phase, collagen molecules are extruded into the extracellular matrix from fibroblasts in a soluble precursor form containing an extra piece which is then 'clipped off' by an extracellular protease, generating the less soluble precursor of crosslinked collagen (Trnavačky, 1974).

One common feature of rheumatic diseases is that the 'target organ' is the connective tissue, which suffers
the consequences of inflammation and often undergoes extensive degeneration. Therefore, many studies of the effect of anti-inflammatory drugs at a biochemical level have been concerned with the influence of such drugs on the connective tissue itself. Biochemical studies of the collagen in granuloma tissue have been undertaken by many workers, motivated with the hope of elucidating the mechanism of action of anti-inflammatory drugs on the proliferating connective tissue. Granulation tissue has been analyzed for the quantity of hydroxyproline as an indicator of the total collagen content. Corticoids have been reported to lower the concentration of hydroxyproline in the tissues of various experimental granulomas (Trnava, et al., 1962) but Jorgensen (1962) failed to find any significant decrease in hydroxyproline concentration, using open-wound technique. Phenylbutazone and salicylates have been reported to have no effect on the hydroxyproline concentration (Trnava, et al., 1962). These findings certainly show that the collagen components of granulation tissue is more susceptible to steroids than the nonsteroidal anti-inflammatory drugs except chloroquine, which reduces the collagen content in a granulation tissue, probably by a marked reduction in the number of synthesizing fibroblasts (Trnava, et al., 1961). Three weeks administration of sodium salicylates decreases the collagen concentration in granulation tissue,
apparently because of enhanced catabolism. These experiments have been performed in granulomas induced by combined mechanical (cotton pellet) and chemical (carrageenin) irritation. Carrageenin itself is known to induce not only proliferation but also degradation of connective tissue (Jackson, 1957), so any catabolic effects of the anti-inflammatory drugs may reinforce that triggered by carrageenin. Some of these conclusions have also been verified when anti-inflammatory drugs are injected directly into the developing carrageenin-induced granuloma (Fukuhara and Zuurufuji, 1969). Steroidal anti-inflammatory drugs (e.g., betamethasone) prevent accumulation of collagen during the formation of granuloma as well as in the granuloma already formed. Salycyl acid and indomethacin affect the collagen concentration only during granuloma formation and have no effect on a preformed granuloma. In our experiments, we followed the procedure of Fukuhara and Zuurufuji (1969) to examine the inhibitory effect of M. cordata root extract on granuloma and examined the effect of M. cordata root extract on the hydroxyproline content of granuloma tissue. It was found that M. cordata root extract significantly decreased the quantity of hydroxyproline content in granuloma tissue, in comparison to the untreated control. Thus it may be concluded that M. cordata root extract causes metabolic alterations of the connective tissues during inflammation.
From all these facts, it is reasonable to conclude that *M. cordata* root extract has a potent anti-inflammatory activity against both proliferative and exudative phases of inflammation.

Subsequently preliminary attempts were made towards purification and identification of the active principle(s) responsible for anti-inflammatory activity.

For purification and isolation of active principle(s), we adopted the general chemical techniques column chromatography. We collected number of residues (fractions) from the extract. The fractions from methanolic extract were first subjected to carrageenin-induced oedema test, if found effective, further attempts in order to get purer products were undertaken. Basis of selecting carrageenin-induced oedema model as a test for anti-inflammatory activity, has already been described earlier.

The extraction procedure for isolation of active principle from crude extract has already been described (Materials and Method). On screening it was found that pooled Fraction, (39-51) possess significant anti-inflammatory activity (Table 21).

This (39-51) fraction is semisolid in nature and
we further proceeded with this pooled fraction (39-51). The fraction (39-51) was chromatographed on TLC silica gel-G-plate and two spots were found to occur out of which one was found to be very feeble in nature.

The fraction (39-51) produced 51.72% & 65.45% inhibition of carrageenan-induced oedema at the dose level of 20 and 40 mg/kg.

The fraction (39-51) was further subjected to physico chemical examinations, e.g., u.v., I.R and chemical tests. From the results of these tests it may be concluded that the active substance contains phenolic group with amides and carbonyl chromophore possibly indicating the presence of active substance(s) similar to flavone.

Most of the nonsteroidal anti-inflammatory agents are potentially ulcerogenic. Accordingly attempts were made to examine the effect of M.cordata root extract on gastrointestinal ulcer models in experimental animals. It was observed that the M.cordata root extract had significant antiulcer action rather than any ulcerogenic action. Several experimental models in animals were examined with respect to both the preventive and healing action of the root extract.

In the preventive test models the root extract (at doses of 50 and 100 mg/kg) significantly inhibited acetyl salicylic acid induced gastric lesions in rats.
by reducing the ulcer index (Table 22 in Results). In steroid-induced gastric ulcers, the M. cordata root extract (50 mg and 100 mg/kg) significantly inhibited the number of ulcers as well as the ulcer index (Table 23 in Results). The effect of M. cordata root extract was examined in another experimental model i.e. serotonin-induced gastric lesion. The root extract (in doses of 50 and 100 mg/kg) was found to cause significant reduction of lesion index (Table 24, Results). The M. cordata root extract (100 mg/kg) on further examination in another model i.e. indomethacin induced gastric lesion was found to cause significant reduction in gastric lesions in the experimental animals (Table 25, Results). However, in the histamine-induced duodenal ulcer model in guineapigs the M. cordata root extract on treatment prior to histamine, produced a significant reduction of no. of ulcers in duodenum and severity of ulcer. There were also inhibition of ulcer index in experimental animals (Table 26, Results).

Thus from the above experiments it was found that the M. cordata root extract was significantly effective in all of the experimental models of preventive test of gastric ulcers in animals.

Impressive results were also obtained in the ulcer healing tests in rats. The administration of M. cordata
root extract (50 and 100 mg/kg) daily for 10 consecutive days caused a significant reduction in ulcer index and significant improvement in healing rate of acetic-acid induced gastric ulcers in rats (Table 27, in Result chapter).

Thus it is evident from the results obtained in the present series of experiments concerning gastro-intestinal ulcers, that the methanolic fraction of the root extract of *M. cordata* significantly prevented gastric lesions induced by non-steroidal anti-inflammatory drugs (NSAID) e.g. acetylsalicylic acid and indomethacin, significantly suppressed the steroid-induced glandular lesions in stomach and serotonin-induced gastric ulcers. Significant prevention of histamine-induced ulcer and the enhancement in ulcer healing rate were also observed with the root extract treatment.

All of the above findings, are suggestive of a potent antiulcer activity of the *M. cordata* root. However, it is interesting at the moment to report that the *M. cordata* root extract having potent anti-inflammatory activity also simultaneously possess anti-ulcer activity, as most of the anti-inflammatory drugs are reported to be potentially ulcerogenic. However, similar antiulcer action of anti-inflammatory compounds from different plant sources e.g. nimbidin from *Azadirachta*
indices (Pillai and Sathakumari, 1984), Kaemferol from Rheum procumbens (Goei, et al., 1988) and anti-inflammatory compounds from Phulnes indicus (Pal and Nagchoudhury, 1989) have already been reported. Carbocenerol sodium also possess anti-ulcer activity (Okabe, et al., 1976; Pillai and Sathakumari, 1984). So this finding is very important from clinical standpoint of view since anti-ulcer effect of M. cordata seems to be an added major advantage of the drug along with anti-inflammatory activity.

In course of our investigations we have also examined the general pharmacodynamics of M. cordata root extract. First, the action of the root extract on central nervous system was examined. The root extract was found to cause alteration of behaviour pattern and caused reduction in locomotor activity when the experimental animals became remarkably quiet after treatment with the root extract. For further examination concerning the actions of the extract on central nervous system the effect of the root extract (100 mg/kg) was examined on spontaneous motility and significant reduction of spontaneous motility in mice was found to occur (Table 29, Figure 33 in Results). The M. cordata root extract (25, 50 and 100 mg/kg) significantly potentiated the pentobarbital-induced sleeping time in a dose dependent fashion (Table 30, Figure 34, Results)
and also produced significant reduction of normal body temperature which was found to occur at 1st hour after administration of root extract (Table 31, Figure 35, Results). All of the above findings are suggestive of a CNS depressant action of the root extract. It may be recalled here that the root extract of M.cordata produced significant antipyretic activity against yeast-induced pyrexia in rats as well. Simultaneous reduction of normothermia and as well pyrexia as observed in this case is not surprising since similar action is known to occur with chlorpromazine type of drugs (Baldessarini, 1985). However, the M.cordata root extract (upto a dose of 500 mg/kg) did not offer any protection against convulsions induced by pentylenetetrazole and strychnine in mice (Table 36, in Result chapter). The root extract although was found to have a significant action against acetic acid-induced writhing, could not be found to possess any such action (upto a dose of 500 mg/kg) in two other experimental models of algesia i.e. tail clip test and caudal immersion test. So it can be concluded that the root extract is effective against inflammatory pain but its analgesic action is not mediated through CNS.

The M.cordata root extract was found to selectively antagonize secondary conditioned response (Table 33,
Results) and also demonstrated antagonism to amphetamine-induced group toxicity in mice (Table 32 in Result chapter). The selective inhibition of the root extract to SCR is not surprising since similar action is known to occur with other psychoactive compounds e.g. Meprobamate and Ansyclonol (Maffi, 1959).

Accordingly basing on these observations (sleeping time potentiation, reduction of normal body temperature, SCR) which suggest pharmacological property of the M.cordata root extract to be quite similar to the other tranquilizing drugs, the effect of M.cordata root extract was further decided to be investigated on certain other characteristic actions of psychopharmacological agents e.g. Exploratory behaviour pattern, Muscle relaxant activity and Electroshock-induced fighting in mice.

The root extract (50 and 100 mg/kg) produced a significant decrease in exploratory behaviour pattern as evident from the results of head dip test (Table 37a, Figure 36b, Result) and T-maze tests (Table 38, Fig.37, Result). The root extract (100, 200 and 300 mg/kg) also produced significant reduction of residual curiosity in groups of mice (Table 39, Result) and this is also suggestive of decrease in exploratory behaviour pattern. Furthermore, in the head dip test when
the root extract was administered in mixtures in combination with amphetamine, a significant increase in head dips occurred (Table 37b, Fig.36a, Results). Both reduction of exploratory behaviour on treatment with M.cordata root extract alone as well on treatment with mixtures of root extract and amphetamine are not surprising and this is in conformity with the actions known to occur with other tranquilizing drugs (Borr et al., 1971).

The M.cordata root extract also significantly inhibited the electroshock-induced fighting in mice (Table 44, Results), indicating that the capability of root extract to antagonize the aggressive behaviour pattern. Inhibition of aggressive behaviour pattern is also another important pharmacological property of the psychoactive agents (Tedeshi et al., 1959).

In tests concerning muscle relaxant activity, the M.cordata root extract (500 mg/kg) was found to produce significant effect in the chimney test (Table 43, Results) while the root extract (up to a dose of 500 mg/kg) did not show any action in the rotarod (Table 40), 30° inclined screen test (Table 42, Results) and traction test (Table 41, Results). Moreover, the root extract (up to dose of 500 mg/kg) did not offer any protection against strychinine-induced convolution indicating the possible absence of any muscle relaxant
activity of the *M. cordata* root extract. Thus the results of the test on the central nervous system with *M. cordata* root extract seem to be a little paradoxical at the moment as although the other characteristic properties of psychoactive substance e.g. reduction of exploratory behaviour pattern and inhibition of aggressive behaviour are demonstrable with *M. cordata* root extract with the absence of any muscle relaxant activity in it, since muscle relaxant activity is another characteristic of the psychopharmacological agents.

However, on the basis of the above findings it can be concluded that *M. cordata* root extract possess a potent CNS-depressant action, mostly similar to that of psychopharmacological agents. However, it is difficult at the moment to indicate the exact nature and category of such action of the root extract, which requires further investigations before any definite conclusion can be drawn on this aspect. The effects of *M. cordata* root extract was also examined on blood pressure of anaesthesized cats. However, no action of the root extract of *M. cordata* could be observed on blood pressure with *M. cordata* root extract observed on at least in the doses employed (i.e. upto 10 mg/kg, i.v.).
On smooth muscle preparations of guineapig's ileum and rat uterus, no significant action (i.e. spasmodic or relaxant) could be observed to occur on treatment with *M. cordata* root extract. The *M. cordata* root extract did not alter the response of Ach (acetylcholine), 5 HT or histamine in guineapig ileum as well as no alteration was found to occur with the response of 5HT in rat uterus. No action of root extract could be found to occur on rat phrenic nerve - diaphragm preparation.

*M. cordata* root extract has neither any cholinergic nor anti-cholinergic effect as it did not affect the responses of acetylcholine on cat blood pressure. The root extract has also no antihistaminic or anti-serotonin activity as evident from the results in cat blood pressure. Moreover, it did not alter the effect of adrenaline-induced response in this preparation. Naturally it may be stated that *M. cordata* root extract has a potent anti-inflammatory and anti-ulcer effect in addition to a potent CNS depressant activity with no demonstrable effect on blood pressure, smooth muscle and neuromuscular preparation (at least in the doses employed).

From the present investigation we may conclude that the *M. cordata* root extract possess a potent anti-inflammatory activity, although such activity on some
parameters of inflammation is somewhat lesser than
the commonly used non-steroidal anti-inflammatory
agents while it is free from the undesirable toxic
side effects as it possess anti ulcer activity and
is practically devoid of any toxic manifestations
that are commonly associated with commonly used non­
steroidal anti-inflammatory drugs. Over and above
to this the M.cordata root extract also possess signi­
ficant CNS depressant activity reminiscent of psycho­
active agents.

ED50 of the M.cordata root extract in carrageenin­
induced oedema model was found to be 48.41 mg/kg but
no mortality was found upto 1500 mg/kg of M.cordata
after administration of as well as it produced no
other toxic manifestations in acute toxicity studies.
The dose ranges that have been proved to elicit anti­
inflammatory effect did not produce any toxic mani­
festations except reduction in frequency of movement
which may be due to its CNS depressant activity (Table
16a, in Result chapter).

The aim of the current investigations was concerned
with a detailed study of the anti-inflammatory effect
of M.cordata root extract with a view towards develop­
ment of an effective anti-inflammatory drug which
should be relatively nontoxic as compared to the cur­
rently used drugs. As the *A.cordata* root extract possesses potent anti-inflammatory property and is free from toxic manifestations there is reasonably good prospect of this plant being considered as a promising anti-inflammatory agent and the results of the present study obviously warrants further detailed investigations with respect to the pharmacological and chemical angles with a view towards ultimate development of an efficacious and superior anti-inflammatory drug in future.

Suggested further investigations are as follows:

1) Separation of pharmacologically active ingredient(s).

2) Chemical characterisation of the separated fractions.

3) Studies on understanding the precise mechanism of anti-inflammatory action of both methanolic fraction of *A.cordata* root extract as well as the active fraction(s).