A

Project work on

"ANALGESIC, ANTI-INFLAMMATORY AND ANTI-DIARRHOEAL ACTIVITY OF *MALVASTRUM COROMANDELIANUM*."

By

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Under the guidance of
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Dept. of Pharmacology

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LIST OF ABBREVIATIONS USED

ATP	-	Adinosine tri phosphate
Ca^{2+}	-	calcium ion
CAM	-	cell adhesion molecules.
cAMP	-	cyclic Adinosin mono phosphate
COX	-	cyclo oxygenase
cGMP	-	Cyclic Guanosin Mono phosphate
CPCSEA	-	Committee for the Purpose of Control and supervision of experiments on animals.
DNA	-	deoxyribonucleic acid
E	-	Evaluative-cognitive
EDRF	-	endothelium derived relaxing factors.
ETEC	-	*Enterotoxigenic E.coli*.
GABA	-	Gamma amino butyric acid.
gm	-	gram.
GRK	-	G-protein-regulated receptor kinases.
h	-	hour.
HCO^{3-}	-	bicarbonate ion.
HEMC	-	Hydro alcoholic extract of *Malvastrum coromandelianum*.
5-HT	-	5-hydroxy tryptamine.
5-HTPETE	-	5-hydroperoxy ecosatetranoic acid.
IL	-	interleukin.

i.p.	-	intra peritoneal.
IU	-	International units
K^+	-	potassium ion
kg	-	kilogram
L	-	Large diameter afferent fibers.
LT	-	Leukotrienes.
mg	-	milligram.
MRI	-	Magnetic Resonance Imaging.
N	-	no. of animals.
Na^+	-	Sodium ion
NMDA	-	N-methyl-D-aspartate
NO	-	Nitric oxide.
NOS	-	nitric oxide synthetase.
ns	-	not significant.
NSAID	-	Non steroidal anti-inflammatory drugs.
P	-	Probability.
PAF	-	platelet activiting factor.
PAG	-	periaqueductal gray matter.
PDGF	-	Platelet-derived growth factor
PET	-	Posistron Emission Tomography.
PG	-	prostaglandin.
PGHS	-	prostaglandin H synthatase.
po	-	per oral.

RA	-	rheumatoid arthritis
ROS	-	Reactive oxygen species
RVM	-	rostral ventromedial medulla.
S	-	Small diameter afferent fibers
SEM	-	Standard error of mean.
SP	-	substance P.
SG	-	substantia gelatinosa
T	-	transmission cells
TNF	-	tissue necrosis factor
TX	-	Thromboxane
%	-	Percentage
μ	-	mu receptor
δ	-	delta receptor
κ	-	kappa receptor

ABSTRACT

In order to scientifically apprise some of the anecdotal, folkloric, ethnomedical uses of *Malvastrum coromandelianum*, the present study was undertaken to examine the antinociceptive, anti-inflammatory and antidiarrhoeal properties of Broom weed's Hydroalcoholic extract (HEMC). The antinociceptive effects of HEMC were evaluated by hot-plate and tail-flick method. Anti-inflammatory effect was evaluated by carrageenan induced paw edema; while anti-diarrhoeal effect was evaluated by castor oil induced diarrhoea, charcoal meal test and PGE_2 induced diarrhoea. Morphine (5 mg/kg), Diclofenac (100 mg/kg), loperamide (3 mg/kg) and atropine (0.1mg/kg) were used as standard drugs for comparison. HEMC was used in 200 and 400 mg/kg. HEMC produced significant antinociceptive effects against thermally induced pain. It also significantly inhibited carrageenan induced acute inflammation, and caused dose related anti-diarrhoeal effect. Results suggest that it may act centrally and may inhibit the PGE_2 to give anti-inflammatory and anti-diarrhoeal effects. Result of charcoal meal test also suggests its anti-muscarnic activity.

Key words: *Malvastrum coromandelianum*; hot-plate; tail-flick; paw edema; castor oil diarrhea; charcoal meal; PGE_2; gate control theory; neuromatrix.

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1. INTRODUCTION

1.1 Pain

Pain is a warning signal that helps to protect the body from tissue damage. Potentially damaging stimuli activate and sensitize certain primary afferent nerve cells. The activity of these nerve cells projects to the spinal cord and from there to the brain, giving rise to the sensation of pain. Clinically, the sensation of pain elicits varying degrees of suffering and depression depending on its duration and patient's psychosocial environment [1].

Types of pain

Acute pain is defined as short-term but extreme pain that comes on quickly but last only for a brief period of time. Acute pain is the body's warning of present damage to tissue or disease. It is often fast and sharp followed by aching pain. Acute pain is centralized in one area before becoming somewhat spread out. This type of pain responds well to medications[2].

Chronic pain was originally defined as pain that has lasted 6 months or longer. It is now defined as pain that persists longer than the normal course of time associated with a particular type of injury. This constant or intermittent pain has often outlived its purpose, as it does not help the body to prevent injury. It is often more difficult to treat than acute pain. The experience of physiological pain can be grouped according to the source and related nociceptors (pain detecting neurons)[3].

The experience of physiological pain can be grouped according to the source and related nociceptors (pain detecting neurons).

Somatic pain originates from ligaments, tendons, bones, blood vessels and even nerves themselves. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, poorly-localized pain of longer duration than cutaneous pain; examples include sprains and broken bones[4].

Visceral pain The density of visceral nociceptors is <1% in comparison with somatic afferents and the cortical mapping of visceral afferents is also less concentrated. Therefore, visceral pain is poorly localized, diffuse and often in the midline, with the exception of joints and the mesentery.

The qualitative nature of the pain is also different because the viscera are sensitive to distension. It also appears that afferent fibers respond in a graded fashion to intensity of stimulation rather than to individual stimulating methods. Visceral pain also exhibits spatial summation, so that if a large area is stimulated, the pain threshold is lowered, this does not occur in cutaneous nociception. Visceral pain can also be referred to a site far away from the source of stimulation. It is often segmental and superficial and frequently shows hyperalgesia[4].

Cutaneous pain is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin and due to the high concentration of nerve endings, produce a well-defined, localized pain of short duration. Examples of injuries that produce cutaneous pain include paper cuts, minor cuts, minor (first degree) burns and lacerations[5].

Neuropathic pain or "neuralgia", can occur as a result of injury or disease to the nerve tissue itself. This can disrupt the ability of the sensory nerves to transmit correct information to the thalamus and hence the brain interprets painful stimuli even though there is no obvious or known physiologic cause for the pain[6].

Phantom limb pain is the sensation of pain from a limb that has been lost or from which a person no longer receives physical signals. It is an experience almost universally reported by amputees and quadriplegics[7].

Pathways of pain[4]

Nociception is conveyed from the periphery to the brain by an adaptable and dynamic pathway. The pathway is transmitted and modulated at three levels: the peripheral nociceptor, the spinal (dorsal horn of the cord) and the supraspinal (brain).

Peripheral activation

Most pain originates after tissue damage. The release of inflammatory mediators from tissues, immune cells and sympathetic and sensory afferent nerve fibers results in an 'inflammatory soup' bathing the nociceptors (**Fig. 4**). These chemicals can directly activate or sensitize the high-threshold nociceptors to activation by low intensity stimuli. This results in primary hyperalgesia. Some mediators act directly on ion channels in the membrane (protons, 5-hydroxytryptamine) but most bind to membrane receptors and act via regulatory intermediates (G-proteins, second messengers) to produce changes in membrane ion channels or enzymes. These inflammatory mediators also interact to form a complex process of events, which change the short-term function of sensory afferent fibers and can alter gene transcription. This can produce long-term changes in cellular biochemistry, receptor and transmitter production.

Spinal level

The dorsal horn of the spinal cord is the site where complex interconnections occur between excitatory and inhibitory interneurons and the descending inhibitory tracts from the brain. The second-order neurons are of two types: nociceptive specific (in laminae II and III), which respond selectively to high threshold nociception and wide dynamic range or convergent neurons (in laminae V and VI), which respond to a range of inputs.

Neurotransmitters at the dorsal horn

Many neurotransmitters play a role in nociceptive transmission in the dorsal horn. The transmitters fall into two groups; excitatory amino acids and neuropeptides. They activate the ionotropic α-amino-3-hydoxy-5-methyl-4-isoazolepropionate and neurokinin-1 receptors, respectively, to produce ionic depolarization of the cell. They also activate a metabotropic receptor, which results in the production of secondary cellular messengers that prime the N-methyl-D-aspartate receptor to be activated. As a result, the excitatory amino acids can then stimulate the N-methyl-D-aspartate receptor, resulting in sustained depolarization. This receptor is pivotal in the maintenance of spinal cord hyperalgesia and therefore is a strong potential target for analgesia. N-methyl-D-aspartate receptor antagonists (ketamine) reduce allodynia and hyperalgesia and enhance opioid analgesia, but can cause hallucinations, sedation and amnesia.

Many neuropeptides are involved in the nociceptive process. Some enhance nociception, such as substance P acting at the neurokinin-1 receptor, neurokinin A and B, cholecystokinin, calcitonin gene-related peptide, bombesin and vasoactive intestinal

peptide. Others are antinociceptive, such as somatostatin, galanin, γ-aminobutyric acid and glycine. All are theoretical targets for modulation, but are not useful clinical agents.

Analgesic drugs targeted at the spinal cord level include local anaesthetics (produce non-specific conduction blockade) and opioids (act on opioid receptors). The endogenous opioids (enkephalin, endorphin, dynorphin A) are rapidly inactivated by peptidases, so exogenous opioids (morphine, fentanyl) must be given. The effects of opioids can be potentiated by α_2-agonists, such as endogenous noradrenaline in the descending inhibitory pathways or exogenous clonidine. Clonidine is limited by sedation, bradycardia and hypotension. Cholinergic agonists (neostigmine) enhance noradrenergic-mediated analgesia. Midazolam produces segmental spinal analgesia, probably via the benzodiazepine receptor, which forms a complex with the γ-aminobutyric acid receptor.

Supraspinal level

The supraspinal function in nociception is beginning to be explored with the advent of non-invasive imaging, such as functional MRI and PET. The perception of pain is associated with changes in activity of the thalamus, primary and secondary cortex and particularly the anterior cingulate cortex.

Various regions of the brain are involved with descending inhibition. These pathways originate at the level of the cortex and thalamus and are mediated via relay stations in the brainstem, such as the periaqueductal grey matter, nucleus raphe magnus and locus coeruleus subcoeruleus complex. The inhibitory pathway then descends the spinal cord via the dorsal columns and terminates at the dorsal horn where neurotransmitters (noradrenaline, 5-hydroxytryptamine) and the endogenous opioids are released to provide antinociception. The three receptors (mu, delta, kappa) play a role in

the ascending pathways but the mu and delta receptors are mainly responsible in the descending component.

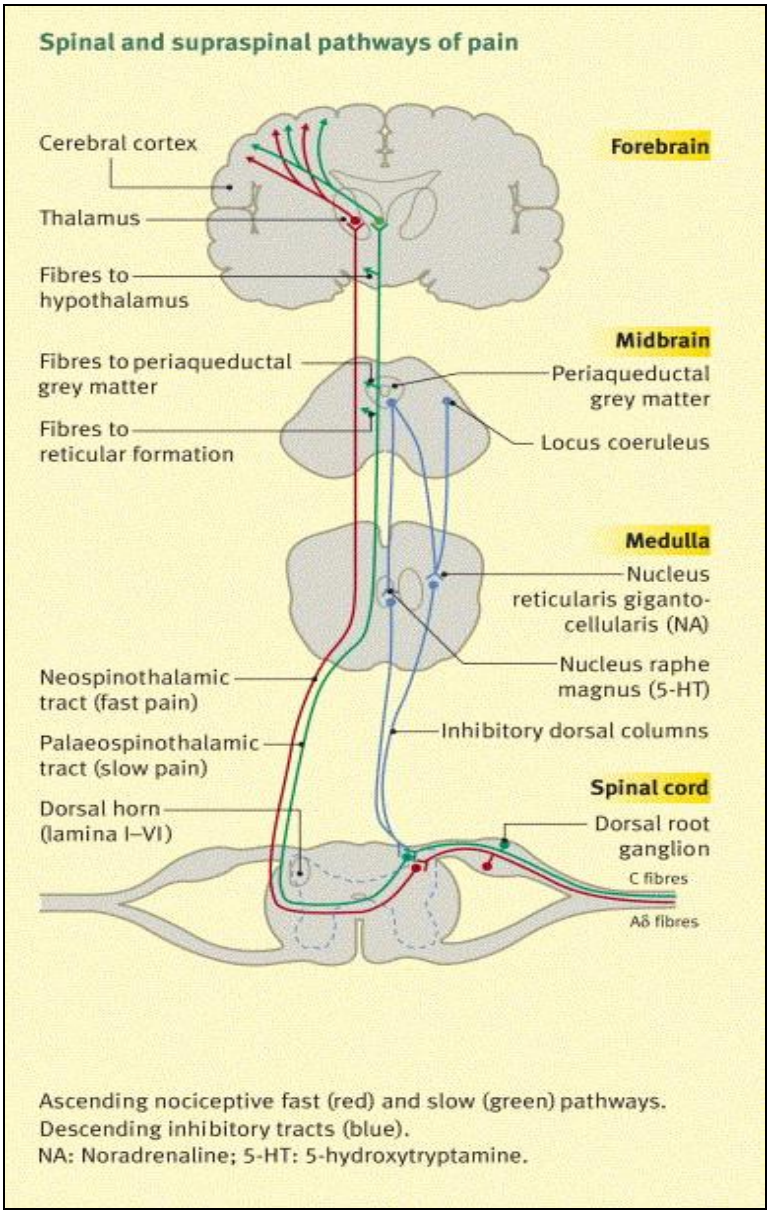


Fig. 1. Spinal and supraspinal pathways of pain[4].

Synthesis and release of noradrenaline and 5-hydroxytryptamine are increased by opioids and they in turn enhance the action of opioids. This may be the mechanism by which the antidepressants and tramadol work as analgesics.

Antinociceptive systems[8]

Opioids activate peripheral, spinal and supraspinal opioid receptors. To date, four different groups of opioid receptors have been identified (μ, δ, κ, ORL-1). In addition, eight isoforms (μ_{1-3}, δ_{1-2}, κ_{1-3}) and numerous subtypes have been pharmacologically characterised. The opioid receptors mediate their effects via an activation of guanine-nucleotide-binding protein (G-proteins), particularly but not exclusively pertussis toxin-sensitive $G_{i/o}$-protein.

Modulation of membrane potential

The β/γ-subunit of G-proteins leads to a K^+-efflux (K_{IR}) and to the closing of voltage-gated Ca^{2+}-channels leading to hyperpolarisation and reduced neuronal excitability. Possible mechanisms of antinociceptive opioid effects are summarized in **Fig. 2.**

Deactivation of adenylate cyclase

A $G_{i/o}$-protein mediated activation of opioid receptors inhibits adenylate cyclase and, consequently, causes a decrease of intracellular cyclic adenosine monophosphate (cAMP), which can also lead to hyperpolarisation and inhibition of neurotransmitter release such as glutamate and substance P (SP) at peripheral, spinal and supraspinal levels.

Receptor trafficking

After activation, the opioid receptor becomes phosphoralised by G-protein-regulated receptor kinases (GRK) and is thereby separated from the G-protein. As a result, the receptor increases its affinity for the cellular protein arrestin and the subsequently activated receptor–arrestin complex can initiate endocytosis. Following internalisation, the receptor is either 'recycled' and will re-express at the cell surface or it will be degraded. Via internalization and re-expression of receptors, the opioid-receptor bond becomes intermittently detached and initiates other adaptive intracellular processes that result in tolerance development. This theory explains observations that the μ-agonist Morphine does not possess a significant capacity for receptor internalization but exhibits a high potential for tolerance development.

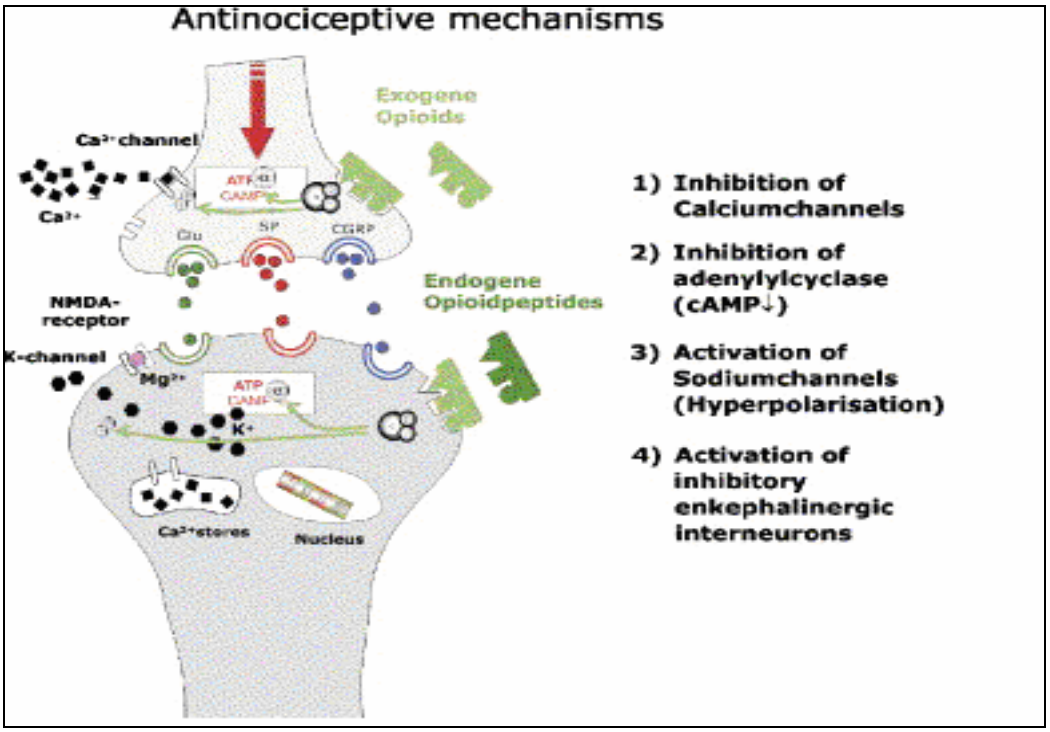


Fig. 2. Schematic diagram of antinociceptive mechanisms mediated by μ-agonists[8].

Descending inhibition

Descending inhibition originates in the periaqueductal gray matter of the midbrain (PAG) and the rostral ventromedial medulla oblongata (RVM). In the RVM, three classes of neurons can be discerned: Off-cells are inhibited by painful stimuli, On-cells increase their firing rate upon painful stimulation and neutral cells do not respond to painful stimuli. Off-cells and On-cells project onto dorsal horn neurons to inhibit and facilitate, respectively, the synaptic transmission of nociceptive inputs. The central analgesic effect of μ-agonists is attributed to an inhibition of On-cells and an activation of Off-cells.

In addition to direct opioid receptor effects, affinity to or interaction with other antinociceptive systems such as GABAergic and glycinergic neurons are important factors to determine an opioid's antinociceptive potency.

1.2 Inflammation

Inflammation is body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects. These effects cause heat, redness (erythema), swelling (edma) and pain, to the area that can result in loss of function. The main symptoms of the body against the inflammatory stimulation or increased body temperature and pain. Inflammation constitutes the body's response to injury and is characterized by a series of events that includes the inflammatory reaction, a sensory response perceived a pain and a repair process[9].

Causes of Inflammation

Some causes of inflammatory reaction are:

- **Infection** – i.e. invasion and multiplication within tissue by organisms. These course, include various bacteria, fungi, viruses and protozoa, which in many instances, cause damage by release of toxins that directly destroy host cells.
- **Trauma** – this includes penetrating injury (e.g. stab wound) blunt trauma, thermal injury (excessive heat or cold) and chemical injury (acid or alkali).
- **Immunologically** mediated (humoral or cellular).
- As a result of the loss of blood supply (**ischemia**).

Signs of the inflammation

- Rubour (redness)
- Tumour (swelling)
- Calor (heat)
- Dolor (pain)

- Function laesa (loss of function)

Systemic effects of inflammation[10]

- Fever
- Chills
- Fatigue/loss of energy
- Headaches
- Loss of appetite
- Muscle stiffness

Pathophysiology

Pathophysiology of the inflammation can be conveniently described under the following two heading

1. Vascular events.

2. Cellular events.

1. Vascular events

Alternation in the microvasculature is the earliest response to tissue injury. These alternations include haemodynamic changes and changes in vascular permeability.

Haemodynamic changes

The initial phase of arteriolar constriction is transient and probably of little importance in acute inflammation. Blood flow may be re-established in 3 to 5 seconds. The subsequent phase of vasodilatation (active hyperaemia) may last from 15 minutes to several hours, depending upon the severity of the injury. There is experimental evidence that blood flow to the injured area may increase up to ten-fold. Progressive vasodialation may, inturn may elevate the local hydrostatic pressure resulting in the transudation of

fluids into extracellular space. This is responsible for swelling at the site of inflammation. Due to the increased vascular permeability concentration of red blood cell increases which causes increase in the blood viscosity. This causes slowing of microcirculation. As blood flow begins to slow again, blood cells begin to flow nearer to the vessel wall, in the plasmatic zone rather than the axial stream. This allows 'pavementing' of leukocytes (their adhesion to the vascular epithelium) to occur, which is the first step in leukocyte emigration into the extravascular space.

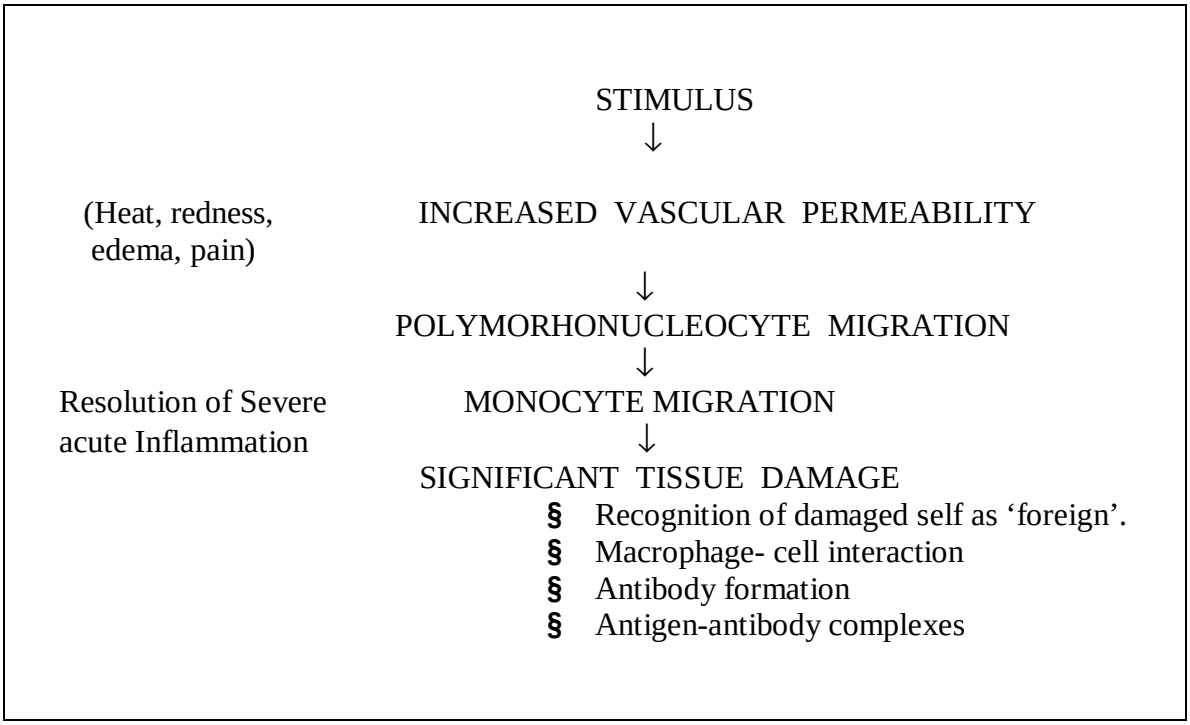


Fig. 3 - Schematic representation of the inflammatory process

Altered vascular permeability

Small blood vessels are lined by a single layer of endothelial cells. In some tissues, these form a complete layer of uniform thickness around the vessel wall, while in other tissues there are areas of endothelial cell thinning, known as fenestrations. The walls of small blood vessels act as a micro filter, allowing the passage of water and

solutes but blocking that of large molecules and cells. Oxygen, carbon dioxide and some nutrients transfer across the wall by diffusion, but the main transfer of fluid and solutes is by ultrafiltration, as described by Starling. The high colloid osmotic pressure inside the vessel, due to plasma proteins, favors fluid return to the vascular compartment. Under normal circumstances, high hydrostatic pressure at the arteriolar end of capillaries forces fluid out into the extravascular space, but this fluid returns into the capillaries at their venous end, where hydrostatic pressure is low. In acute inflammation, however, not only is capillary hydrostatic pressure increases, but there is also escape of plasma proteins into the extravascular space, increasing the colloid osmotic pressure there. Consequently, much more fluid leaves the vessels than is returned to them. The net escape of protein-rich fluid is called exudation; hence, the fluid is called the fluid exudates.

2. Cellular events

The cellular phase of inflammation consist of two processes

A) Exudation of leucocytes

B) Phagocytosis

A) Exudation of leukocytes

The changes leading to migration of leucocytes are as follows

a) Changes in the formed elements of blood

In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. Subsequently, there is slowing or stasis of blood stream. With stasis, changes in the normal axial flow of blood in the microcirculation takes place. Due to slowing the stasis, the central stream of cells widen and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as

margination. As a result of this redistribution the neutrophils of the central column come close to the vessel wall, this is known as pavementing.

b) Rolling and Adhesion

In this, peripherally marginated and pavemented neutrophils stick briefly to the endothelial cells lining the vessel wall or roll over it. This happens as a result of interaction of adhesion molecules on leukocytes and endothelial cells surface. These molecules (cell adhesion molecules = CAM) are expressed by chemical mediation.

c) Emigration

After sticking of neutrophils to endothelium, they begin to squeeze through the wall of the blood vessel to reach the damaged area. This process is known as emigration. Simultaneous to emigration of leukocytes, escape of red cells through gaps between the endothelial cells, diapedesis takes place.

d) Chemotaxis

The chemotactic factor mediated transmigration of leukocytes after crossing several barriers to reach the interstitial tissues is called chemotaxis.

B) Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells. There are two types of cells, which are involved in phagocytosis.

- Polymorphonuclear neutrophils
- Macrophage

Mediators of inflammation

A mediator of inflammation is defined as any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response. The mediation of inflammation comprises an extensive network of interacting chemicals that render the system with a high degree of redundancy. This guarantees that both amplification and preservation of the response can be maintained even if one component or another of the system is dependent on a single mediator.

They originate from plasma cells and in plasma found in inactive stage and must be activated. Microbial products or host proteins trigger production of active mediators. One mediator can stimulate the release of other mediators by target cells (provide mechanism of amplification). Most of the mediators are short lived.

Table 1. Mediators in Inflammation and Pain[11]

Vasodialation	**Increased Vascular Permeability**	**Chemotaxic Leukocyte Activation**	**Fever**	**Pain**	**Tissue Damage**
PG, NO	Vasoactive amines, C3a and C5a (through liberating Vasoactive amines from	C5a, LTB4 Chemokines (e.g. IL-8)	IL-1, IL-6, TNF-α, PG	PG Bradykinin	Neutrophil And Macrophage Products Lysosomal Enzymes Oxygen

	cells), Bradykinin, LTC4, D4, E4, PAF				Metabolite, NO

Table 2. Some of the mediators in acute inflammation and their effects.

Mediators	**Vasodilatation**	**Vascular Permeability**	**Chemotaxis**	**Pain**
Histamine	++	↑↑↑	-	-
Serotonin	+/-	↑	-	-
Bradykinin	+++	↑	-	+++
Prostaglandin	+++	↑	+++	+
Leukotrienes	-	↑↑↑	+++	-

Some of the mediators and their role

I) Vasoactive amines

This includes histamine and serotonin that are believed to be the primary mediators in the immediate active phase of increased permeability and cause vasodialation and increased vascular permeability by causing endothelial cells to round up and increase vesiculovacuolar transfer of fluids. These chemicals are stored within the cells for immediate release.

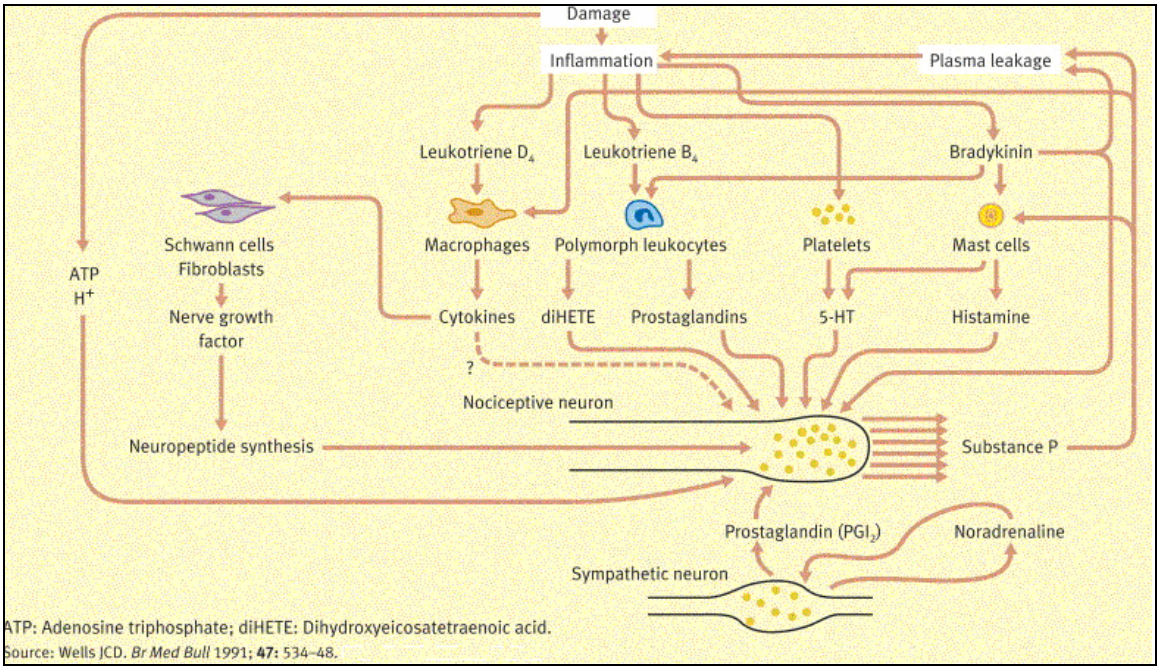


Fig. 4. Inflammatory mediators[4].

Histamine

It is extensively distributed in tissues, the main source begins the mast cells that are normally present in the perivascular connective tissue. It is also present in granules of basophiles and in platelets (some species). Histamine is important mainly in early

inflammatory responses and in immediate IgE mediated hypersensitivity reactions. It is preformed and stored in granules heparin. Histamine is important in the immediate active phase of increased vascular permeability, promotes contraction of extravascular smooth muscles in the bronchi and stimulates stromal cells to synthesize and release eotaxins, (chemotaxins for eosinophils). The following agents can stimulate release of histamine from mast cells.

- Physical injury, mechanical trauma, heat, chemical agents.
- Snake venoms, toxins, bile salts, ATP.
- Immune reactions involving binding of antibodies to mast cells.
- Fragments of complement called anaphylatoxins (C3a and C5a).
- Histamine-releasing factors from neutrophils, monocytes and platelets.
- Cytokines (interleukin-1, IL-8), Neuropeptides.

Serotonin

It is a vasoactive mediator, present in platelets and some mast cells (not in humans). Serotonin has the following characteristics:

- Act primarily on venules
- Important in early phase of acute inflammation
- It is released from mast cells, basophils and platelets

II) Plasma proteins

Three various and interrelated systems important in inflammatory response are found within plasma. These systems include the complement, kinin and clotting systems.

Complement system[12]

Consist of activating and effectors sequences consisting of 20 protein components most common in plasma. The system functions both in innate and adaptive immunity. This plasma protein acts primarily as a defense mechanism against microbial agents. The product of complement activation are involved in vascular permeability (C3a, C5a and C4a (lesser extent) cause histamine release from mast cells (referred to as anaphylatoxins), Chemotaxis - C5a chemoattractant for neutrophils, monocytes, eosinopils and basophils, opsonization prior to phagocytosis and lysis of target organisms. C3 and C5 are the most important inflammatory mediators. Plasmin and lysosomal enzymes in the inflammatory exudates can also activate the proteins.

The Kinin System

The Kinin system generates vasoactive peptides from plasma proteins called kininogens by the action of specific proteases called kallikreins. This system results in the ultimate release of the vasoactive non peptide bradykinin. Bradykinin is a potent vasodilator, Increases vascular permeability, contracts smooth muscles, produce pain, stimulates release of histamine from mast cells and activities the arachidonic acid cascade.

Clotting system[13]

The clotting system and inflammation are intimately connected. The intrinsic clotting system is a sequence of plasma proteins that can be activated by Hageman factor (factor XII produced in lever and circulating in inactive form). The final phase of cascade is conversion of fibrinogen to fibrin. This fibrin binds to protease activated receptor on platelets and endothelial cells. This binding produces mobilization of P-selectin,

production of chemokines, induction of cyclooxygenase2, thus stimulate the production of prostaglandins causing inflammation.

III) Arachidonic Acid metabolites

Arachidonic acid is mainly stored in cell walls (any cell), from which it is mobilized largely by the action of enzyme phospholipase A2. They are short lived, extremely potent and formed in almost every tissue in the body. They are involved in most types of inflammation and most present anti-inflammatory therapy is based on altering their synthesis in the body. These lipid mediators are thought of as short-range hormones that are formed rapidly and exert their effects locally and then are inactivated. Oxygenated arachidonic acid derivatives have roles in a variety of biologic and pathogenic processes, only one of which is inflammation. This enzyme is stimulated due to any injurious stimuli. Corticosteroids act by inhibiting this enzyme. Arachidonic acid is further metabolized by cyclo-oxygenase and lipo-oxygenase.

Cyclo-oxygenase Pathway

This pathway produces prostaglandins. PGE_2 and PGI_2 causes erythema and increased blood flow, PGE_2 and PGF_2 causes intense local pain, PGE_1 causes itching, PGE_2 associated with production of fever, NSAIDs exert their anti-inflammatory effects by inhibiting cyclo-oxygenase enzyme.

Lipo-oxygenase Pathway[14]

The most important metabolites are leukotrienes, so designated because of their conjugated 'triene' chain and their initial isolation from leukocytes. Leukotriene B_4 is a potent chemotactic agent. Leukotrienes C_4, D_4, E_4 are very potent vasoconstrictors; they also act as potent mediators of increased vascular permeability on venules only. These

leukotrienes are up to 1000 times as potent as histamine in producing increased vascular permeability.

Lipoxins

Leukocytes, primarily neutrophiles produce intermediates in lipoxin synthesis that are converted to lipoxins by platelets interacting with leukocytes. Lipoxins A_4 and B_4 are generated.

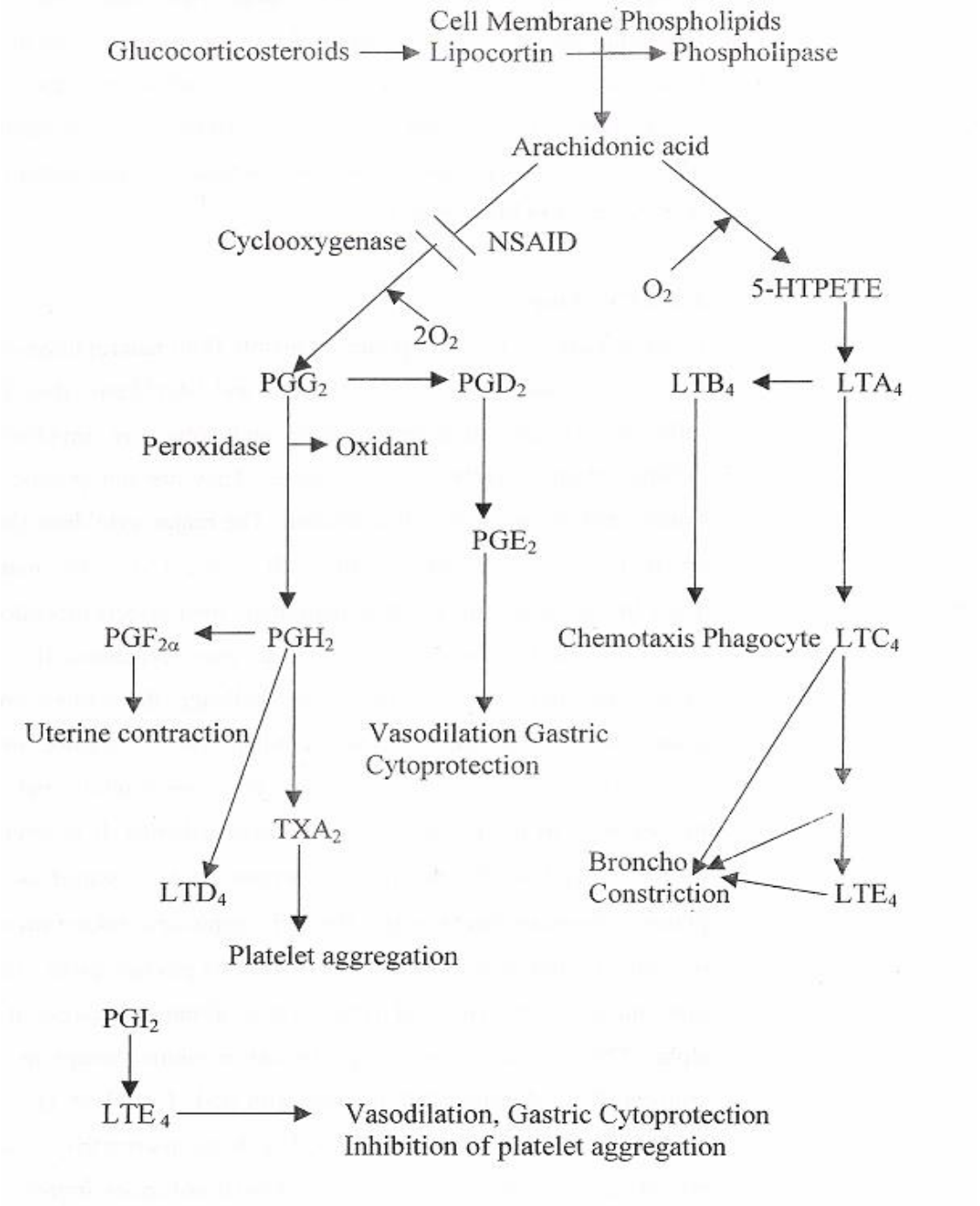


Fig. 5: Biosynthetic pathway of eicosanoids[15]

IV) Platelet activating factor (PAF):

These are bioactive phospholipids produced by a variety of cells, including platelets, basophils, mast cells, neutrophils, monocytes, macrophages and endothelial cells following stimuli. PAF produces its effect via a single G-protein-coupled receptor: Platelet aggregation and release, bronchoconstriction and vasoconstriction, vasodilatation and increased vascular permeability (to histamine), increased leukocyte adhesion to endothelium, leukocyte chemotaxis, degranulation and oxidative burst.

V) Cytokines

Cytokines are the peptides produced mainly from macrophages and lymphocytes but also from other leukocytes, endothelial cells and fibroblasts; they function as regulators of inflammatory and immune reactions and some are involved in multiplication and differentiation of cells repair processes. They are not produced constitutively but are synthesized on cell activation. The major cytokines that mediate inflammation are IL-1, IL-10 and TNF (α and β). IL-1 and TNF serve many biological properties. Their important actions in inflammation are their effect on endothelium, leukocytes and fibroblast and induction of systemic acute phase reaction. IL-1 molecules are produced in infection and injury or on antigenic challenge. It regulates the systemic inflammatory response stimulating the synthesis of acute phase proteins and the increase in the blood neutrophils and causing fever by altering the set point of the temperature in the hypothalamus. It synergies with Tumor necrosis factor alpha (TNF-α) for many of its actions and its synthesis is stimulated by TNF-α (**Tumor Necrosis Factor**-a); is the early response cytokine involved in the pathogenesis of various inflammatory conditions besides its physiological role in normal host defense mechanism. Monocytes and

lymphocytes primarily produce it. Tumor necrosis factor alpha (TNF-α) is potential target of anti-cytokine in patients with rheumatoid arthritis (RA). Because TNF-α along with IL-1, IL-8, IL-6 is overproduced in the joints of RA patients. However, TNF-α has been particularly associated with the joint inflammation of this disease. Interleukin-10 enhances immune activity by stimulating proliferation, activation and chemotaxis of CD 81 t cells, enhancing natural killer cell activity and cytokine production. IL-10 also inhibits the production of granulocyte macrophage colony stimulating factor, which cause neutrophil and monocyte/macrophage activation. Interleukin-10 can down regulate the induction of nitric oxide synthase in macrophage cells. Since nitric oxide is an inflammatory mediator, IL-10 can attenuate inflammation via this pathway[16].

VI) Growth factors:

Platelet-derived growth factor (PDGF) and transforming growth factor-β may be chemotactic to leukocytes and mesenchymal cells and have activities resembling those of cytokines. Growth factors are particularly important in tissue regeneration and repair.

VII) Nitric oxide (NO):

It is an important mediator in acute and chronic inflammation. It is generated via oxidation of terminal guanidine nitrogen atom of L-arginine by enzyme nitric oxide synthetase (NOS) (Three major isoforms of NOS have been identified). NO is a potent vasodialator. It acts by relaxing vascular smooth muscles-endothelium derived relaxing factors (EDRF) during its involvement during inflammatory response. This action is related to its increased vascular permeability and edema through changes in blood flow. It has very limited sphere of action due to its short half-life, which is in milli sec to sec. Furthermore NO has shown to increase PG production in-vitro. In addition NO can also

react with superoxide anion to form peroxynitrite, a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage. In addition to vascular smooth muscle relaxation, NO reduces platelet aggregation and adhesion.

Reactive oxygen species (ROS):

ROS are associated with the inflammatory response and frequently they contribute to the tissue damaging effects of inflammatory reactions. On the other hand they are important mediators of programmed cell death induced by TNF-α. ROS formation and degradation are key components of the metabolism of aerobic organisms. Certain levels of ROS are required for normal cell functions, but if in surplus, they will cause oxidative stress. ROS like superoxide, hydrogen peroxide and lipid hydro-peroxides can regulate the activities of several kinases, transcription factors, cell death machinery and proteins such as COX-2 and NOS. Neutrophils play a crucial role in the development and manifestation of inflammation and they are the major source of free radicals at the site of inflammation[17].

Apoptosis in inflammation:

In order to cease the inflammatory response the infiltration of inflammatory effector cells has to be stopped and the existing population of pro-inflammatory cells must be eliminated without provoking the release of pro-inflammatory mediators. The apoptotic process achieves this normally involves activation of the proteolytic cascade of caspase family proteases. An important determinant of the resolution of inflammation is apoptotic removal of leukocytes with subsequent clearance of the apoptotic bodies by phagocytosis. Therefore, the development of therapeutic strategies aimed at inducing apoptosis in rheumatoid arthritis and other chronic inflammatory disorders is an attractive

goal since (I) reduced apoptosis may play an important role in the pathogenesis of chronic inflammation and (II) promotion of apoptosis in the chronically inflamed tissue may have an anti-inflammatory effect by itself. This goal can be addressed in two ways: (1) by including apoptosis in the inflammatory effector cells or (2) by inhibiting the anti-apoptotic mechanisms of these cells. Several anti-apoptotoic molecules have been identified. Among them, transcriptional regulators such as p53, NF-B and Stat3, have been suggested to regulate apoptosis most prominently. Phagocytosis triggers macrophage release of CD178 (Fas ligand), an event that may lead to the induction of the apoptosis of bystander leukocyte. This will happen only towards the end of a successful inflammatory response since freshly stimulated T-cells are resistant towards CD95 (Fas, Apo-1)-triggered apoptotic death[18].

Medications used to treat inflammatory diseases[10]

There are many medications available to decrease joint pain, swelling and inflammation and hopefully prevent or minimize the progression of the inflammatory disease. The medications include:

- Non-steroidal anti-inflammatory drugs (NSAID)
- Corticosteroids (such as prednisone)
- Anti-malarial medications (such as hydroxychloroquine)
- Other medications including methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate

1.3 Diarrhoea

Diarrhoea occurs world-wide and causes 4% of all deaths and 5% of health loss to disability. It is most commonly caused by gastrointestinal infections which kill around 2.2 million people globally each year, mostly children in developing countries. The use of water in hygiene is an important preventive measure but contaminated water is also an important cause of diarrhoea. Cholera and dysentery cause severe, sometimes life threatening forms of diarrhoea. The infectious agents that cause diarrhoea are present or are sporadically introduced throughout the world. Diarrhoea is a rare occurrence for most people who live in developed countries where sanitation is widely available, access to safe water is high and personal and domestic hygiene is relatively good. World-wide around 1.1 billion people lack access to improved water sources and 2.4 billion have no basic sanitation. Diarrhoea due to infection is widespread throughout the developing world. In Southeast Asia and Africa, diarrhoea is responsible for as much as 8.5% and 7.7% of all deaths respectively. Each year there are approximately 4 billion cases of diarrhoea worldwide[19].

Diarrhoea is the passing of increased amounts (more than 300g in 24 hours) of loose stools. It is often caused by a virus or bacteria and can be acute (short term) or chronic (long term more than two or three weeks).

Most people are affected by diarrhoea at some time in their lives. It is often accompanied by stomach pains, feeling sick and vomiting. It is usually due to consumption of drinking water contaminated with bacteria, undercooked meat and eggs or inadequate kitchen hygiene - in other words, an infection.

Causes of diarrhoea[20]

A few of the more common causes of diarrhoea include the following

- **Bacterial infections:** Several types of bacteria consumed through contaminated food or water can cause diarrhoea. Common culprits include *Campylobacter, Salmonella, Shigella and Escherichia coli (E. coli).*
- **Viral infections:** Many viruses cause diarrhoea, including rotavirus, Norwalk virus, cytomegalovirus, herpes simplex virus and viral hepatitis.
- **Food intolerances:** Some people are unable to digest food components such as artificial sweeteners and lactose—the sugar found in milk.
- **Parasites:** Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhoea include *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium*.
- **Reaction to medicines:** Antibiotics, blood pressure medications, cancer drugs and antacids containing magnesium can all cause diarrhoea.
- **Intestinal diseases:** Inflammatory bowel disease, colitis, Crohn's disease and celiac disease often lead to diarrhoea.
- **Functional bowel disorders:** Diarrhoea can be a symptom of irritable bowel syndrome.

Symptoms of diarrhoea[21]

- Frequent, watery motions.
- Loss of appetite.
- Nausea, vomiting.
- Stomach pains.

- Fever.
- Dehydration.

Warning signs[21]

- Blood in the motions.
- Pus in the motions (yellow mucus).
- Inability to drink liquids because of vomiting.
- Dehydration - symptoms include excreting small amounts of dark urine, drowsiness, dry mucous membranes and thirst. Dehydration as a result of diarrhoea is a particular risk for young children and the elderly.
- Pronounced drowsiness due to dehydration or intoxication.
- Acute diarrhoea in infants.
- Acute diarrhoea in very old people.

Diagnosis of diarrhoea[20]

Diagnostic tests to find the cause of diarrhoea may include the following:

- **Medical history and physical examination:** The doctor will ask patient about their eating habits and medication use and will examine you for signs of illness.
- **Stool culture:** A sample of stool is analyzed in a laboratory to check for bacteria, parasites or other signs of disease and infection.
- **Blood tests:** Blood tests can be helpful in ruling out certain diseases.
- **Fasting tests:** To find out if a food intolerance or allergy is causing the diarrhoea, the doctor may ask you to avoid lactose, carbohydrates, wheat or other foods to see whether the diarrhoea responds to a change in diet.

- **Sigmoidoscopy:** For this test, the doctor uses a special instrument to look at the inside of the rectum and lower part of the colon.
- **Colonoscopy:** This test is similar to a sigmoidoscopy, but it allows the doctor to view the entire colon.
- **Imaging tests:** These tests can rule out structural abnormalities as the cause of diarrhoea.

Types of Diarrhoea[22]

There are at least five types of diarrhoea: secretory diarrhoea, osmotic diarrhoea, motility-related diarrhoea, inflammatory diarrhoea and traveller's diarrhoea.

- **Secretory diarrhoea**

  Secretory diarrhoea means that there is an increase in the active secretion or there is an inhibition of absorption. There is little to no structural damage. The most common cause of this type of diarrhoea is a cholera toxin that stimulates the secretion of anions, especially chloride ions. Therefore, to maintain a charge balance in the lumen, Sodium is carried with it, along with water.

- **Osmotic diarrhoea**

  Osmotic diarrhcea occurs when there is a loss of water due to a heavy osmotic load. This can occur when there is maldigestion (e.g. pancreatic disease or Celiac disease), where the nutrients are left in the lumen, which pulls water into the lumen.

- **Motility-related diarrhoea**

  Motility-related diarrhoea occurs when the motility of the gastrointestinal tract is abnormal. If the food moves too quickly, there is not enough contact time between the

food and the membrane, meaning that there is not enough time for the nutrients and water to be absorbed. This can follow a vagotomy or diabetic neuropathy.

- **Inflammatory diarrhoea**

  Inflammatory diarrhoea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhoea can be found in this type of diarrhoea. It can be caused by bacterial infections, viral infections, parasitic infections or autoimmune problems such as inflammatory bowel disease.

- **Travellers' diarrhoea**

  Travellers from temperate regions of the world frequently experience diarrhoea four days to two weeks after arriving in certain areas of the world. This illness is called travellers' diarrhoea.

Mechanism of intestinal Ion and Water transport

Water and electrolytes are absorbed as well as secreted in the intestine. Jejunum is freely permeable to salt and water which are passively absorbed secondary to the nutrient (glucose, amino acids etc.) absorption. In the ileum and colon active Na^+K^+ATPase mediated salt absorption occurs, primarily in the mature cells lining the villous tips, water follows iso-osmotically. In addition glucose facilitated Na^+ takes place in the ileum: one Na^+ ion is transported along with each molecule of glucose absorbed. This mechanism remain intact even in sever diarrhoeas.

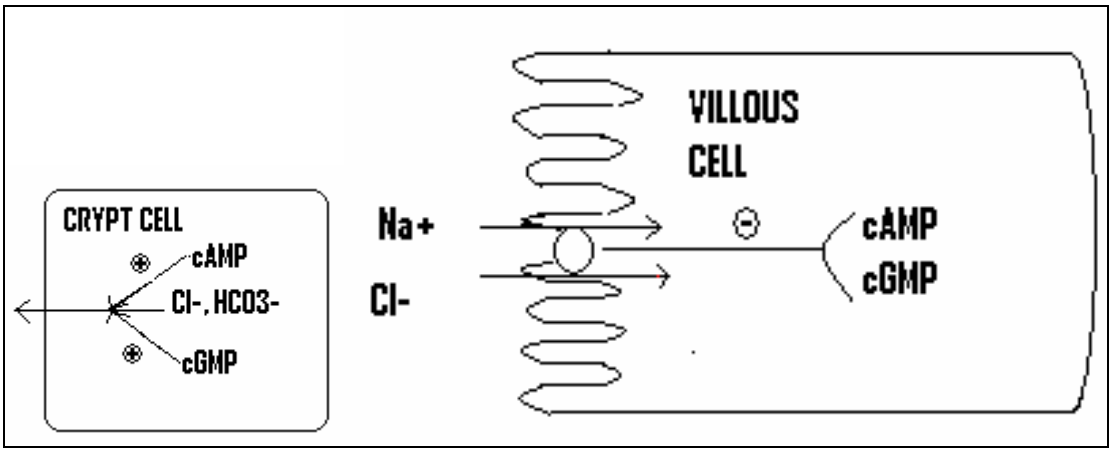


Fig.6. Action of cyclic nucleotides on electrolyte transport of intestinal mucosal cells.

Absorption of Cl^- and HCO_3^- is passive (paracellular) as well as by exchange of HCO_3^- Cl^- (transcellular). Bicarbonate is absorbed also by the secretion of H^+ (similar to that in proximal tubule of kidney) and Na^+ accompanies it. K^+ is excreted in faecal water by exchange with Na^+, as well as by secreation in to mucus and in desquamated cells. The osmotic lode of luminal contents plays an important role in determining final stool volume. When non absorbable solutes are present in disaccharidase deficiency (which occurs during starvation), the stool water is increased. Inhibition of Na^+ K^+ ATPase and structural damage to mucosal cell (by rota virus) causes diarrhoea by reducing absorption.

Intracellular cyclic nucleotides are important regulators of absorptive and secretary process (**Fig. 6**). Stimuli enhancing cAMP or cGMP cause net loss of salt and water, both by inhibiting NaCl absorption in villous cells and by promoting anion secretion (Na^+ accompanies) in the crypt cells which are primarily secretory. Many bacterial toxins, eg. Cholera toxin, exotoxin elaborated by *Enterotoxigenic* E.*coli* (ETEC), *Staph. aureus*, *Salmonella* etc. activate adenylyl cyclase which enhances secretion that reaches its peak after 3-4 hrs and persist until the stimulated cells are shade

in the normal turnover i.e. 36 h after single exposure. Concurrent inhibition of absorption adds to the rate of salt and water. Prostaglandins (PGs) and intracellular Ca^{2+} stimulate the secretory process. All acute enteric infection produces secretory diarrhoea. The heat stable toxin (ST) of ETEC, *Clostridium diffcile* and *E. histolytica* cause accumulation of cGMP which stimulates anion secretion (less potent than cAMP) and inhibit Na^{+} absorption.

Diarrhoea associated with carcinoid (secreting 5-HT) and medulary carcinoma of thyroid (secreting calcitonin) is mediated by cAMP. Excess of bile acid also caused diarrhoea by activating adenylyl cyclase.

Traditionally hyper motility of bowel has been ascribed a crucial role in diarrhoea. However, changes in intestinal motility are now thought to be of secondary importance and may be due to fluid accumulation in lumen. Decreased segmenting activity in the intestine may promote diarrhoea by allowing less time for absorptive processes[23].

Plant

Fig.7 Image of *Malvastrum coromandelianum*

Latin Name: *Malvastrum coromandelianum*

Family: Malvaceae.

Common Names: False mallow, Broom weed, clock plant[24].

Synonyms: *Malva tricuspidata, Malvastrum tricuspidatum*[24]*, Malva coromandeliana, Malva coromandilina Linn,. Malva tricuspidata R. Br, Malva luzonica Blanco Malva tricuspidatum A. gray*[25]

Parts Used: Whole Plant[26]

Description: An erect branching herb or under shrub, 0.6-0.9 m high. Stem petiole and main nerves on the lower surface of the leaf stellately hairy, with the hairs few branched, ascending or descending; hairs on the blade often simple. Leaves up to 6.5 cm long, ovate or ovate lanceolate, irregularly toothed. 5 nerved at the base, nerves impressed above, prominent beneath; petiole up to 18 mm long, not swollen near the base, flattened or slightly channeled above, densely hairy; stipules 5 mm long linear hairy. Peduncles 12 mm long. Bracteoles 3, linear about half the length of the calyx. Calyx campanulate, cleft half way down lobes 5, triangular, acute. Corolla 12 mm across, pale yellow, exceeding the calyx. Staminal tube anthriferous to the top without sterile teeth. Styles as many as carpel; stigmas capitates. Carpels 8-12, reniform with 3 projecting spines, bristly between spines[27, 28].

Distribution: The plant is native to America. Introduced in India and now found in Maharashtra[29], Tamilnadu[30], Punjab, Orissa and Bengal[31, 32]. It is also found throughout Karnataka[33].

Habitat / ecology: It is a weed of waste places and plantations. It is found in waste ground, by roadsides, in abandoned city lots etc. Locally abundant near sea level as a

naturalized weed in waste places, gardens, cane fields, open fields and along roadsides. At low altitudes, mainly in areas with seasonal rainfall[34].

Propagation: Seed

Traditional uses-: In West Indies plant is considered to be emollient. The leaves are applied to inflammed sores and wounds. The flowers are given as a pectorial and diaphoretic[27]. It is used as anti-pyretic. It is having smooth muscle relaxant property[35]. It is used as an anti-inflammatory, analgesic and anti-dysentric[36,37].

CHAPTER 2
OBJECTIVS

2. OBJECTIVES

1) Preparation of different extracts of aerial parts of *Malvastrum coromandelianum.*

2) Preliminary phytochemical screening of different extracts.

3) To study the analgesic activity of hydroalcoholic extract of *Malvastrum Coromandelianum* by-

   A) Tail-flick method.

   B) Hot plate method.

4) To study the anti-inflammatory activity of hydroalcoholic extract of *Malvastrum Coromandelianum* by-

   A) Carrageenan induced paw oedema.

5) To study the anti-diarrhoeal activity of hydroalcoholic extract of *Malvastrum Coromandelianum* by-

   A) Castor oil induced diarrhoea.

   B) Charcoal meal test.

   C) PGE_2 induced diarrhoea.

CHAPTER 3
REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Antinociceptive property of the aerial parts of *Malvastrum coromandelianum* in the 0.6% acetic acid-induced writhing test in mice was reported by Y. S. R. Reddy et al. The effects of acetone extract (200 mg/kg, p.o.) being comparable with acetylsalicylic acid (100 mg/kg, p.o.)[38]

Jiang T et al. were obtained a virus isolate Y160 from *Malvastrum coromandelianum* showing yellow vein symptoms in Baoshan, Yunnan province of China. The complete nucleotide sequence of DNA-A was determined, it contains 2747 nucleotides and has typical genomic organization of a begomovirus. Comparisons show that the total DNA-A of Y160 has the highest sequence identity (82.2%) with that of Malvastrum yellow vein virus-[Y47] (AJ457824), while less than 77.6% identities are found when compared with the other begomoviruses[39].

In June 2004 Kallappa M. et al. reported that *Malvastrum coromandelianum* contain palmitic acid (22.7%), palmitoleic acid (2.4%), stearic acid (2.7%), oleic acid (14.6%), linoleic acid (37.0%), malvalic acid (10.5%) and sterculic acid (10.1%)[40].

The use of *Malvastrum coromandelianum* as antipyretic and smooth muscle relaxant was reported by S. A. Dhanukar, R et al[35].

Yajuan Qian, et al. were observed that in association with heterologous begomoviruses, such as TYLCCNV or *Malvastrum yellow vein virus*, the DNAΔC12β vector could still effectively induce transgene and endogenous gene silencing in tobacco plants[41].

The International Association for the Study of Pain (1994) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or, described in terms of such damage."[42]

The gate control theory was proposed in 1965 by Melzack and Wall to explain the highly variable and non-linear relationship between injury and response to pain. According to this the peripheral sensations are transmitted via large diameter (L) fast conducting nerves and via small diameter (S) slow conducting fibers. The L nerve fibers are easily activated by minimal stimulation like touch. Such stimulation on reaching the spinal cord, activates the first transmission cell (T_1) and also activates the collateral cells possibly in the substantia gelatinosa (SG). The SG cells by a negative feedback system then inhibit T_1cells and decrease their output reaching the higher centers. If the peripheral stimulus is more intense, not only L nerves but also S nerves will be activated. The effect of the S nerve stimulation on SG cells is to augment rather than diminish T_1 activity and thus increase their output reaching the higher centers (fig 8). It is also probable that there are both inhibiting and facilitating mechanisms which operate via the higher centers on T cell activation. This mechanism (the ST gate control) causes the sensory input to be decreased or augmented depending on the relative activity of L and S nerves which in turn depends on the intensity of peripheral stimulation by noxious stimuli[43,44].

This is the working mechanism behind transcutaneous electrical nerve stimulation in pain control. The gate-control theory does not explain secondary hyperalgesia, which results in a larger receptive field with exaggerated responses to noxious stimulation and increased spontaneous discharge rate. This situation is usually temporary and resolves

with tissue healing, but it occasionally becomes permanent and produces a chronic pain state.

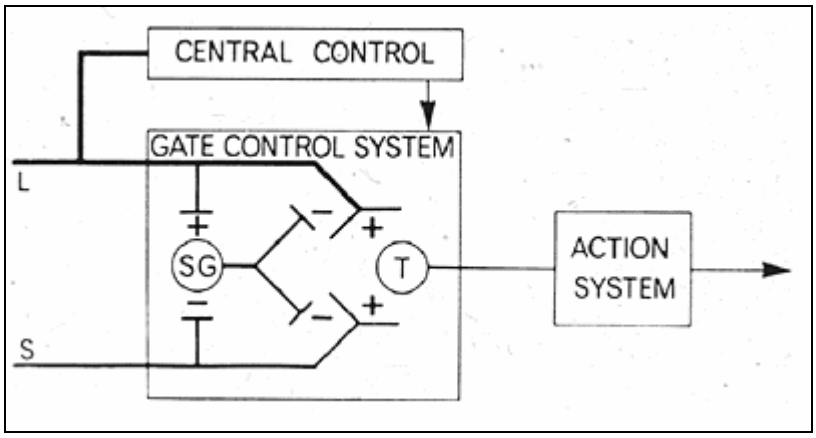


Fig.8. The Gate Control Model. Large-diameter afferent fibers (L) stimulate the substantia gelatinosa (SG) and the transmission cells (T) in lamina 4. The SG cells reduce the membrane potential of afferent terminals, thus producing presynaptic inhibition. Small-diameter afferent fibers (S) also stimulate the transmission cells but inhibit SG cells and thus turn off the existing presynaptic inhibition[45].

Melzack has since moved beyond his initial gate control theory to a reconceptualization known as the “neuromatrix.” The neuromatrix theory of pain posits that pain is a multidimensional experience produced by characteristic "neurosignature" patterns of nerve impulses that are generated by a widely distributed neural network - the "body-self neuromatrix" - in the brain. It is a parallel and serial process that can be thought of as an expansion of the central control processes in the original gate control theory. The central control processes would encompass cognitive-evaluative, motivational-affective and sensory-discriminative systems.

Multiple factors act on the neuromatrix and contribute to the output "neurosignature." These factors include sensory inputs; visual and other sensory inputs that influence cognitive interpretation; phasic and tonic cognitive and emotional inputs; intrinsic neural inhibitory modulation; and the activity of the stress-regulation system (endocrine, autonomic, immune and opioid systems). Pain may be triggered by sensory inputs, but may also be generated independently of them. Thus, pain could be produced by the output of a widely distributed neural network in the brain, rather than directly by nociceptive stimuli.

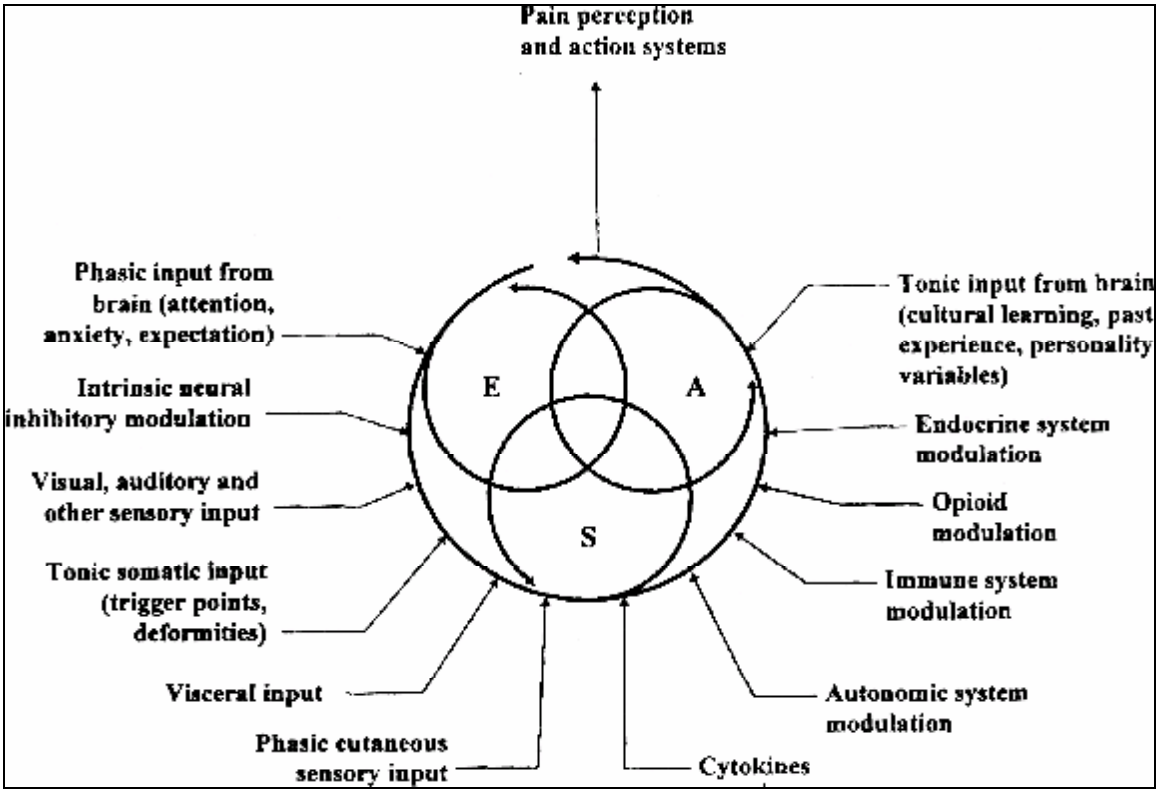


Fig.9. The body-self neuromatrix: Three parallel processing networks: (S) Sensory discriminative; (A) Affective-motivational; (E) Evaluative-cognitive.

The neuromatrix theory is still evolving and the brain functions and mechanisms in this schema need to be elucidated. The pain theories have traveled from the peripheral pain fibers to pain as a "feeling" state. This is paralleled by the evolution of the treatment of pain in pharmacology and psychology. The science of pain continues to explore the genetic, endocrine and immune systems, all of which may contribute to the neuromatrix[44,46].

A wide variety of chemicals can activate the nociceptor terminal. These include endogenous inflammatory mediators (e.g. hydrogen ions, bradykinin and serotonin) as well as exogenous agents (e.g. capsacin and formalin). Individual molecular transducer for many of these agents has been identified. Some of these transducers are cation channels and hence can directly depolarize the nerve ending. Others (e.g. bradykinin and some 5-HT receptors) excite sensory neurons by activating ion channels indirectly via second messenger cascades (including G-proteins and kinases) [47].

The identification of mechanisms that transduce noxious non-chemical stimuli has only recently become a subject of intensive research. It has recently been shown that there are both cell membrane and intracellular thermally dependant mechanism that lead to activation in primary afferent nociceptors[48,49]. Both a vanilloid and vanilloid like receptor, found in small diameter rat sensory neurons, have been showen to produce heat activated non specific cation currents when expressed in non neuronal cells[50]. Another, likely different, mechanisms involves thermal activation of non specific cation channel in small diameter neurons[47] which appears to be calcium dependent[51].

The aminoglycoside, gentamicin, a blocker of some mechanogated ion channels, blocks the initial burst of C-fiber nociceptor activity evoked by mechanical stimuli[52] and

another aminoglycoside, neomycin, blocks the slowly adapting activity of mechanoreceptors[53]. Cultured visceral afferents exhibit a stretch-activated calcium influx that can be blocked by gadolinium[54]. That a mechanically activated ion channel, in fact, underlies these effects is supported by the observation of stretch-activated single channels in dorsal root ganglion neurons and by genetic evidence of touch-cell-specific ion channels in the nematode, *Caenorhabditis elegans*[55] and of a related ion channel in presumptive mechanoreceptive neurons in *Drosophila melanoguster* [56].

The release of inflammatory mediators during inflammation, tissue injury produces local vasodialation, plasma extravasation and sensitization of nociceptors. 5–HT one of the typical inflammatory mediator and also important neurotransmitter, is released from platelets at the site of tissue injury and is known to play an important role in peripheral nociceptive mechanism and inflammation. Studies using rat knee-joint perfusion model have demonstrated that 5-HT produces significant synovial plasma extravasation, which appears to be mediated by $5HT_{2A}$ receptors located on postganglionic sympathetic neuron terminals[57].

Sympathetic neuron terminals are actively involved in the local inflammatory pathways and sensitization of nociceptors[58,59]. Upon stimulation sympathetic terminals release in addition to norepinephrin, several cotransmmitors including prostaglandins(PGs). PGs are generated from arachidonic acid, an essential fatty acid found in the cell membrane. The first rate limiting enzyme in PG synthesis pathway is cyclooxygenase which has two (COX also named as prostaglandin H synthatase, PGHS) isoforms: COX1 (PGHS1) and COX2 (PGHS2)[60,61]. PGs exert their biological action by binding to thee multiple receptors, which are classified EP, DP, FP, IP and TP subtypes

on the basis of sensitivity the endogenous prostanoid ligands PGE_2, PGD_2, PGF_{2a}, PGI_2 and thromboxane A_2 (TXA_2), respectively. EP receptors are further divided into four subtypes: EP_1, EP_2, EP_3 and EP_4[62]. PGs, especially PGE_2, are significant inflammatory mediators.

Study by Xie and et al. shows that multiple, but selective, subtypes of PGs mediate synovial plasma extravasation produced by 5-HT and suggest that PGs act downstream of 5-HT in the inflammatory cascade[63].

Macrophages are involved in most of inflammatory reactions. Nitric oxide (NO) was suggested to play a role in these reactions, but the available data are contradictory. In some reports nitric oxide was found to be pro-inflammatory[64,65], while other authors observed its anti-inflammatory effects as well[66,67]. The overall effect of NO depends on the type of the inflammation. Nitric oxide is produced from L-arginine by nitric oxide synthase (NOS) isoenzymes, particularly by the inducible isoform in macrophages. The activity and expression of NOS II and arginase can be stimulated in peritoneal macrophages *in vitro* by injecting inflammatory agents into the peritoneal cavity[68].

Recently, it was shown that ligation of CD40, a molecule whose counter-receptor CD154 is expressed predominantly on activated T-cells, can trigger COX-2 expression and PGE_2 synthesis by human lung fibroblasts[69]. Endothelial cells present in chronically inflamed tissues have been shown to express CD40[70] and employ CD40-mediated signaling mechanisms to acquire a proinflammatory phenotype in vitro[70,71]. CD40-CD154 interactions have been shown to induce secretion of various proinflammatory cytokines such as IL-1*b*, IL-6, IL-8 and TNFα from endothelial cells, fibroblasts, monocytes and other cell types in vitro[72].

Diarrhoea can be caused by an increased osmotic load within the intestine, excessive secretion of the electrolytes and water into the intestinal lumen, exudation of proteins and fluid from the mucosa and altered intestinal motility, resulting in rapid transit.

Castor oil converts in to active metabolite recinolic acid[73], which stimulate the peristaltic activity of small intestine, leading to change in electrolyte permeability of intestinal mucosa[74]. Its action is also associated with stimulation of endogenous prostaglandins[75].

Recinolic acid induces irritation and inflammation of intestinal mucosa, leading to prostaglandin release which inturn provokes stimulation of secretion, there by preventing reabsorption of NaCl and water[76].

Prostaglandins are important regulators of gastrointestinal fluid secretion[77,78]. The cells in the underlying mucosa, such as macrophages, can also respond to bacteria with increased PGE_2 production[79]. The production of PGE_2 (and possibly other prostaglandins) by cells other than intestinal epithelial cells likely contributes to the host secretary response at later stages of the infection.

Early studies demonstrated that in the diarrhoeal effect of castor oil NO, may be involved, that increases the permeability of the epithelial layer to the Ca^{2+} ion leading to an increase in intracellular Ca^{2+} enhancement of calmudin stimulation of NO synthetase activity. NO could stimulate the intestinal secretion[80]. Alternatively the effect of castor oil may be attributed to disorder motility and hence to an increase in intestinal transit of intraluminal material. In this condition castor oil could alter coordination of intestinal motility and could promote greater loss of fluid from intestine[81].

The standard drug loperamide produces rapid and sustained inhibition of peristaltic reflex through depression of longitudinal and circular muscle activity. It reduces the daily faecal volume and decreases intestinal fluid and electrolyte loss. The drug also possesses anti-secretary activity presumably through intestinal opiate receptors[14].

The atropine produced a significant reduction in the number of stools and increased intestinal transit time possibly due to its anticholinergic effect[82]. Further, an increase in intestinal transit time with atropine could also result due to reduction in gastric emptying[83].

CHAPTER 4
METHODOLOGY

4. METHODOLOGY

4.1 Collection of the crude drug:

Crude drug, i.e. aerial parts of *Malvastrum coromandelianum* were collected from the forest of Ghulewadi range and dried under shade. The crude drug was authenticated from Botanical Survey of India by PSN Rao, Joint Director of BSI, Pune. The crude drug were subjected to pulverizations and passed through sieve no.40. The powder was packed into a soxhlet apparatus and extracted with petroleum ether (60-80º) for 18 h. The same marc was successively extracted with chloroform and afterwards with hydroalcohol (30:70) for 18 hours. The extracts were dried at 75ºC in water bath for 5 h when a solid mass was obtained in case of hydroalcoholic extract respectively and were stored in airtight containers in refrigerator at below 10ºC. The extracts thus obtained were subjected to phytochemical analysis.

4.2 Phytochemical estimations of the extracts[84,85].

The extracts of *Malvastrum coromandelianum* were subjected to qualitative analysis for the various phytoconstituents. Tests for common phytochemicals were carried out by standard methods.

4.3 Experimental animals

Male albino Wistar rats weighing between 150-235 gm were used. Institutional Animal Ethics Committee approved the experimental protocol l997/c/6/CPCSEA; animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Albino rats were used in this thesis was obtained from the Bioneeds Animal House Dhabas Pet, Tumkur. The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The animals had free access of standard diet and water and housed in a spacious cage for one weak. The composition of the diet are protein 10%, Arachis oil 4%, Fibers 1%, Calcium1%, Vitamin A 1000 IU/ gm Vit D 500 IU/gm.

After procuring the animals kept under standard husbandry conditions as follows:

Room temperature – 26 ± 2^0

Relative humidity – 45 - 55%

12 h light/dark cycle

4.4 List of Various Materials Used

- Anesthetic ether (Karnataka Fine Chemicals, Bangalore).
- Alcohol (Gauri Industries limited, Mandya).
- Morphine tablet (Pravara Rural Hospital).
- Diclofenac (Lupin, Mumbai).
- Carrageenan (Hi-media, Mumbai).
- Loperamide (Micro Lab, Bangalore).
- Activated charcoal (Karnataka Fine Chemicals, Bangalore).
- Atropine (Samarth Pharma, Mumbai).
- PGE_2 (Astra-Zeneca, Bangalore).

4.5 METHODS

4.5.1 Evaluation of analgesic activity by tail flick analgesiometer and hot plate analgesiometer:

4.5.1.1 Tail-flick method[86]

Rats of either sex (150-235gm) were fasted for 18 h. They were then divided into four groups (n=6). Analgesic activity was assessed using the tail-flick assay with an analgesia instrument that uses radiant heat. This instrument is equipped with an electrically heated Cu coil and a 12-s cutoff to prevent damage to the animal's tail. During testing, the lower half of the animal's tail was put just above the heated wire and tail-flick latency was recorded. Each rat served as its own control. Thus, before treatment, its reaction time was determined thrice at 0, 20 and 40 min intervals. The mean of these three determinations constituted the 'initial reaction time'— i.e. reaction time before treatment of the rat. Baseline values for tail-flick latency were determined before drug administration in each animal. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group received standard drug, morphine (5 mg/kg) orally as suspension. The HEMC was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. 30 min after treatment the reaction time was again evaluated at 30, 60, 90,120, 150 and 180 min. This final 'test' mean reaction time value represented 'after-treatment reaction time' (Ta) for each treated rat. These 'test' reaction time value (Ta) was subsequently used to determine percentage protection, using (T_b) as basal reaction time, by applying the formula:

$$\%\text{Protection} = \frac{\text{Test reaction time}}{\text{basal reaction time}} \text{ X } 100 - 100 = \frac{\text{Ta}}{\text{Tb}} \text{ X}100 - 100$$

4.5.1.2 Hot Plate Method[87]

Rats of either sex (150-235gm) were fasted for 18 h. They were then divided into four groups (n=6). Analgesic activity was assessed using the hot plate analgesiometer that uses heated metal plate (with adjustable temperature). A 15-s cutoff to prevent damage to the animal's paw. The temperature of the hot-plate was then regulated to 45±1ºC. Each mouse was placed on the hot-plate in order to obtain the animal's response to electrical heat-induced nociceptive pain stimulus (licking of the forepaws and eventually jumping). The time taken for each mouse to jump out (i.e., reaction time) was noted and recorded in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined thrice at 0, 20 and 40 min intervals. The mean of these three determinations constituted the 'initial reaction time'— i.e. reaction time before treatment of the mouse. Baseline values were determined before drug administration in each animal. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second received standard drug, morphine (5 mg/kg i.p.) as suspension. The HEMC was administered by i.p. route at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. 30 min after treatment the reaction time was again evaluated at 0.5, 1, 2, 3, 6 and 24 h. This final 'test' mean reaction time value represented 'after-treatment reaction time' (Ta) for each treated mouse. This 'test' reaction time value (Ta) was subsequently used to determine percentage protection, using (T_b) as basal reaction time, by applying the formula:

$$\%\text{Protection} = \frac{\text{Test reaction time}}{\text{basal reaction time}} \text{ X } 100 - 100 = \frac{\text{Ta}}{\text{Tb}} \text{ X}100 - 100$$

4.5.2 Evaluation of anti-inflammatory activity:

4.5.2.1 Carrageenan induced paw edema[88]:

Rats of either sex (150-235gm) were fasted for 18 h. They were then divided into four groups (n=6). Before any treatment, volume of the right paw of each animal was determined thrice at 0, 20 and 40 min intervals using a venire caliper. The mean of these three determinations constituted the Vo, basal volume. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group received standard drug, diclofenac (100 mg/kg) orally as suspension. The HEMC was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. 30 min minutes later, paw edema was induced by subplantar injection of 0.1 ml carrageenan (0.1%) into the plantar surface of the right hind paw. 30 min after treatment the paw volume was again measured at 30, 60, 90,120, 150 and 180 min. This final 'test' volume value represented 'after-treatment paw volume' (V_t) for each treated rat. This 'test' mean paw volume value (V_t) was subsequently used to determine percentage protection. These individual records allowed to find out the variation of edema (Vt − Vo) for each group. Percentages of inhibition (*I*%) in each treated group was determined using the following formula:

$$I\% = 100 - [B \times 100]/A,$$

Where *A* is the mean variation of edema (Vt − Vo) for the control group and *B* is the (Vt − Vo) for the treated groups with extracts or compounds.

4.5.3 Evaluation of anti-diarrhoeal activity:

4.5.3.1 Castor oil induced diarrhoea[89]:

Rats of either sex (150-235gm) were fasted for 18 h. They were then divided into four groups (n=6). The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receive standard drug, Loperamide (3 mg/kg) orally as suspension. The HEMC was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. After 60 min of treatment, the animals of each group received 1ml of castor oil orally and the watery faecal material and number of defecation was noted up to 4 h in the transparent metabolic cages with filter paper at the base. Weight of paper before and after defecation was noted.

4.5.3.2 Charcoal meal test[90]:

Rats of either sex (150-235gm) were fasted for 18 h. They were then divided into four groups (n=6). The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receives standard drug atropine (0.1 mg/kg) subcutaneously. The HEMC was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. The animals were given 1ml of 10% activated charcoal suspended in 10% aqueous tragacanth powder p.o., 30 min after treatment. Animals were killed 30 min after charcoal meal administration by inhalation of ether. The abdomen cut off and the small intestine carefully removed. The distance traveled by charcoal plug from pylorus to caecum was measured and expressed as percentage of the distance traveled by charcoal plug for each of animal.

4.5.3.3 PGE_2 induced enteropooling[91]

Rats of either sex (150-235gm) were fasted for 18 h. They were then divided into four groups (n=6). A solution of PGE_2 was made in the 5%v/v ethanol in the normal saline. The first group which served as PGE_2 control, was administered with PGE_2 (100 µg/kg p.o.) only. The second group which served as vehicle control was administered with aqueous 1% tragacanth suspension by oral route. The HEMC was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. Immediately after extract administration PGE_2 was administered. After 30 min following administration of PGE_2, each rat was sacrificed and whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

4.6 Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

CHAPTER 5
RESULTS

5. RESULTS

5.1 Qualitative Chemical Examination

All the extracts were systemically analyzed to detect the presence of the chemical constituents. The result are shown in Table no.3

Table No.3 Results of phytochemical screening *Malvastrum coromandelianum*

Chemical Constituents	Tests	Pet. Ether Extract	Chloroform extract	Hydro alcoholic extracts
(a) Carbohydrates	Molish's test	-ve	-ve	-ve
	Benedicts test	-ve	-ve	-ve
	Feeling's test	-ve	-ve	-ve
(b) Alkaloids	Mayers test	-ve	-ve	-ve
	Wagner's test	-ve	-ve	-ve
	Dragendroff's test	-ve	-ve	-ve
	Hager's test	-ve	-ve	-ve
(c) Glycosides	Modified borntragger's test	-ve	+ve	+ve
	Legal's test	-ve	+ve	+ve
(d) Saponins	Froth test	+ve	+ve	+ve
(e) Phytosterols	Liberman Burchard test	+ve	+ve	+ve
and triterpenoids	Salkowski's test(Steroid)	+ve	+ve	+ve

(f) Fats and oil	Stain test	+ve	+ve	+ve
(g) Phenolic and Tannins	Ferric chloride test	-ve	-ve	+ve
	Lead acetate test	-ve	-ve	+ve
(h) Proteins and amino acids	Milon's test	-ve	-ve	-ve
	Biuret test	-ve	-ve	-ve
	Ninhydrin test	-ve	-ve	-ve
(i) Test for Flavonoids	Shinoda test	-ve	-ve	+ve

1. Pet. ether extract contains saponins, steroids, fixed oils.
2. Chloroform extract contains saponins, steroids, fixed oils, anthracene and cardiac glycosides.
3. Hydroalcoholic extract contains saponins, steroids, fixed oils, anthracene and cardiac glycosides, flavonoids and tannins.

5.2 Effect of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by tail-flick method.

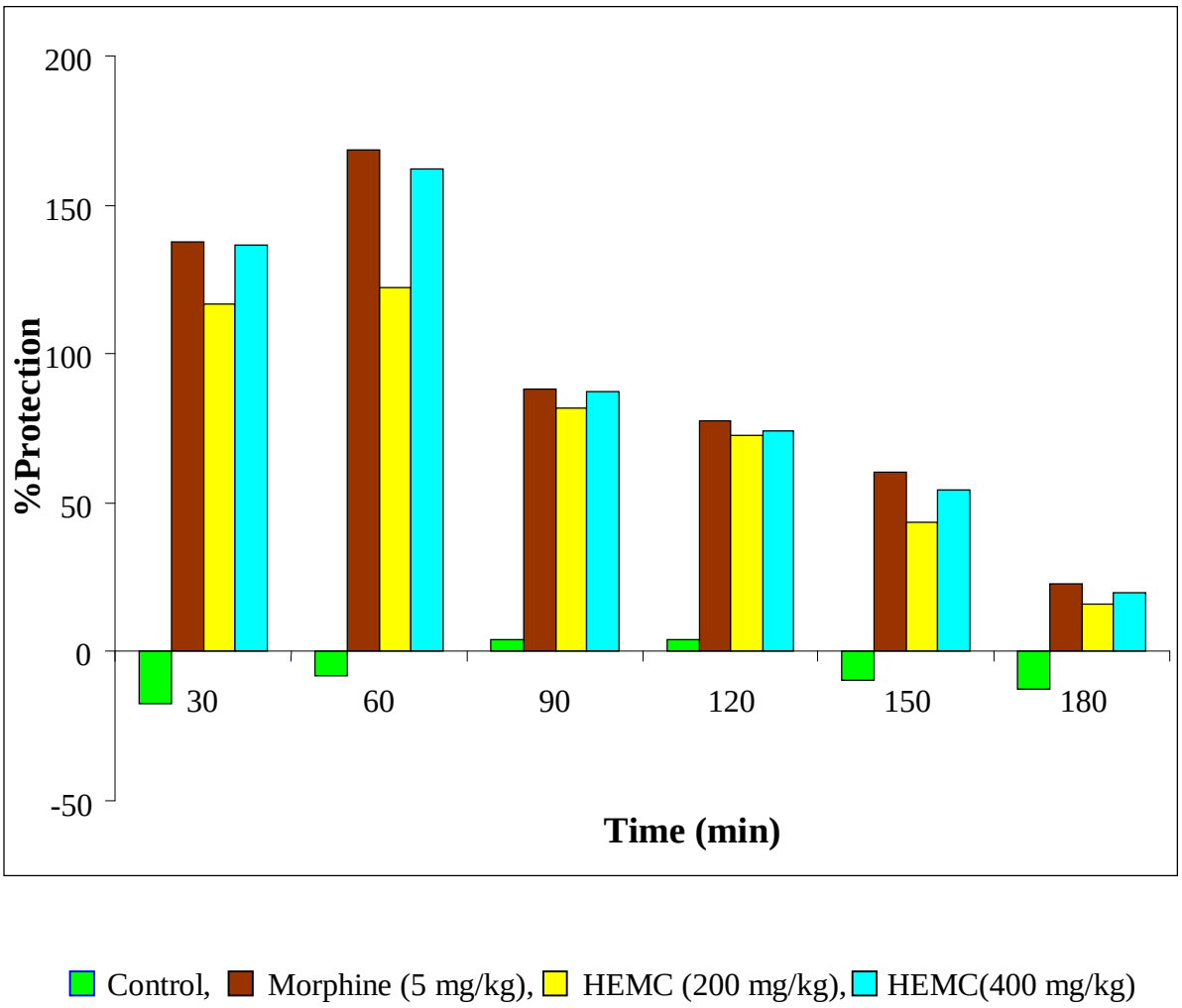
HEMC produced dose dependent analgesic activity significantly ($P < 0.01$) against tail flick method. The antinociceptive effects of the HEMC by the tail flick test in rats are summarized in Table 4. Pretreatment with the extract significantly increased reaction time in comparison to control. At a dose of 400 mg/kg of HEMC exhibited analgesic effect to the same degree as morphine 5 mg/kg up to 150 min (Fig. 10) and at 180 min its effect was significant but lesser than the standard in comparison with control. The peak effect (162.2%) of HEMC 400 mg/kg was shown at 60 min which is almost equal to the peak effect (168.39%) of morphine 5 mg/kg (Fig.10).

Table 4: Evaluation of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by tail-flick method.

Treatment	Increase in reaction time (sec)					
	Different time intervals (min)					
	30	60	90	120	150	180
Control	-1.090 ± 0.51	-0.412 ±0.55	0.178 ± 0.86	0.060 ± 0.73	-0.75 ± 0.76	-0.78 ± 0.48
Morphine (5 mg/kg)	6.146 ± 0.58**	7.379 ± 0.34**	3.829 ± 0.45**	3.476 ± 0.18**	2.651 ± 0.27**	1.064 ± 0.24**
HEMC (200 mg/kg)	4.282 ± 0.82**	5.112 ± 0.71**	3.312 ± 0.70**	3.095 ± 0.44**	1.828 ± 0.49**	0.650 ± 0.41*
HEMC (400 mg/kg)	4.690 ± 0.70**	5.745 ± 0.68**	3.453 ± 0.51**	3.288 ± 0.79**	1.998 ± 0.40**	0.710 ± 0.28*

**=P<0.01= very significant
*=P<0.05= significant
Number of animals (N)=6
Values are expressed as mean±SEM

Fig. 10 Evaluation of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by tail-flick method.



5.3 Effect of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by hot plate method.

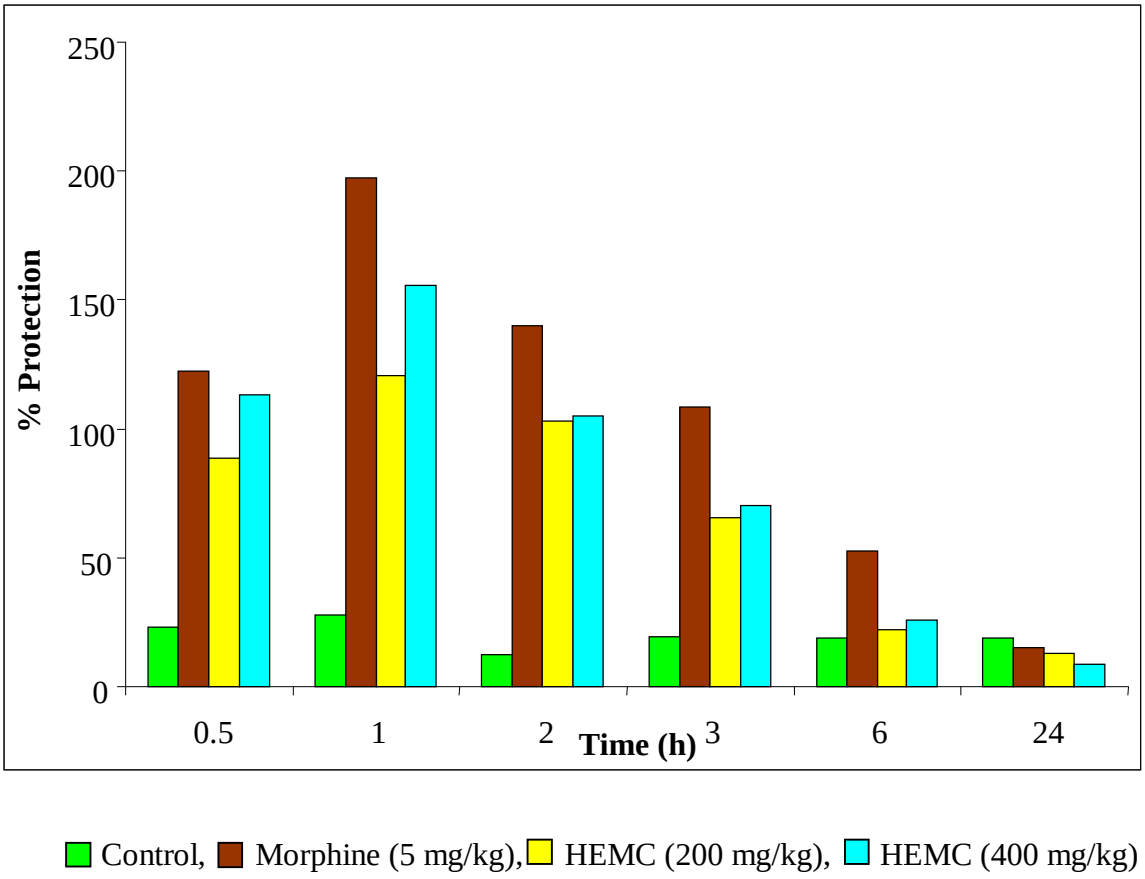
The antinoceptive effect of HEMC by the hot plate method was produced dose dependently on mice and was showed in table 5. At the dose of 200 mg/kg was not significant after 30 min of its administration but its 400 mg/kg dose produced significant ($P<0.01$) effect. The HEMC showed peak effect 120.61% and 155.35% at the dose of 200 mg/kg and 400 mg/kg respectively at 60 min after treatment while morphine was also showed peak effect 197.22% at 60 min (Fig.11) in comparison with control. Comparatively the effect of high dose of HEMC was lesser than the effect of morphine. The HEMC was showed significant analgesic effect up to 3 h after the treatment whereas morphine was showed significant effect up to 6 h.

Table 5: Evaluation of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by hot plate method.

Treatment	Increase in reaction time (sec)					
	Different time intervals (h)					
	0.5	1	2	3	6	24
Control	0.623 ± 0.07	0.704 ± 0.15	0.266 ± 0.16	0.498 ± 0.17	0.503 ± 0.13	0.4842 ± 0.19
Morphine (5mg/kg)	5.493 ± 0.73 **	9.476 ± 0.50 **	6.976 ± 0.64 **	5.241 ± 0.5 **	2.491 ± 0.45**	0.7242 ± 0.19 ^{ns}
HEMC (200 mg/kg)	2.415 ± 0.58^{ns}	3.177 ± 0.49 **	2.618 ± 0.37 * *	1.808 ± 0.24 *	0.493 ± 0.07 ^{ns}	0.2700 ± 0.06 ^{ns}
HEMC (400 mg/kg)	5.281 ± 1.19 **	6.544 ± 0.45 **	4.406 ± 0.25 **	2.729 ± 0.16 **	1.083 ± 0.20 ^{ns}	0.6608 ± 0.31 ^{ns}

**=P<0.01= very significant
*=P<0.05= significant
Not significant (ns)=P>0.05
Number of animals (N)=6
Values are expressed as mean±SEM

Fig. 11 Evaluation of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by hot plate method.



5.4 Antiinflammatory effect of hydroalcoholic extract of *M. coromandelianum*

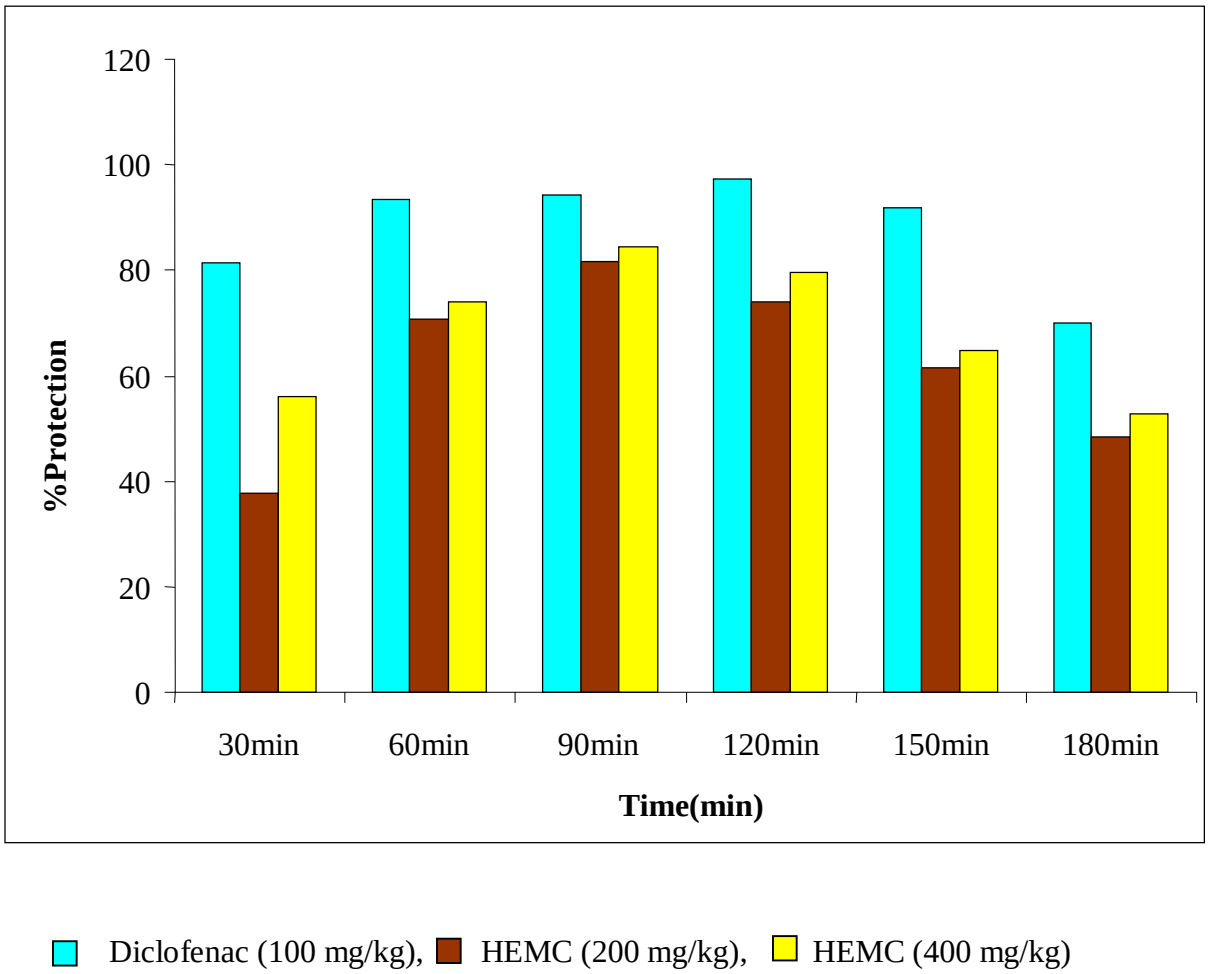
The anti-inflammatory activity of diclofenac sodium 100 mg/kg and HEMC was measured at the dose of 200 mg/kg and 400 mg/kg b.w. against acute paw oedema induced by carrageenan. The average right back paw diameter and percentages of inhibition of edema by the extract in a dose dependent manner and standard drug are shown in table 6. The HEMC produced significant ($P < 0.01$) anti-inflammatory activity and the results were comparable to that of diclofenac sodium (100 mg/kg) as a standard anti-inflammatory drug (Fig.12). The maximum percent inhibition was showed 81.73 and 84.57 at the doses 200 and 400 mg/kg of HEMC respectively at 90 min, while, diclofenac sodium showed 97.38 % at 120 min against acute paw oedema induced by carrageen in comparison to control group.

Table 6: Evaluation of anti-inflammatory activity of hydroalcoholic extract of *M. coromandelianum* by caraggeenan induced paw oedema.

Treatment	Increase in paw size (mm)					
	Different time intervals (min)					
	30	60	90	120	150	180
Control	2.167± 0.33	3.083±0.30	2.917±0.27	3.167±0.25	2.42±0.30	2.33±0.36
Diclofenac (100 mg/kg)	0.40± 0.13**	0.20±0.12**	0.167±0.13**	0.083±0.08**	0.20±0.12**	0.70±0.15**
HEMC (200 mg/kg)	1.350± 0.25^{ns}	0.967±0.21**	0.533±0.14**	0.82±0.12**	0.93±0.14**	1.2±0.09**
HEMC (400 mg/kg)	1.217± 0.27*	0.80±0.26**	0.45±0.18**	0.65±0.18**	0.85±0.18**	1.1±0.23**

**=P<0.01= very significant
*=P<0.05= significant
Not significant (ns)=P>0.05
Number of animals (N)=6
Values are expressed as mean±SEM

Fig. 12 Evaluation of anti-inflammatory activity of hydroalcoholic extract of *M. coromandelianum* by carageenan induced paw oedema.



5.5 Antidiarrhoeal activity of hydroalcoholic extract of *M. coromandelianum*

Castor oil produced watery diarrhoea, which lasted up to 24 h in the vehicle treated control group. The HEMC exhibited pronounced antidiarrhoeal effect in a dose-dependent manner following oral pretreatment on castor oil-induced diarrhoea compared with the control. The extract prolonged the onset time of diarrhoea, 70.83 min and 132.33 min, at the dose of 200 and 400 mg/kg respectively. Although the effect is significant but comparatively it is lesser than the loperamide 3 mg/kg (200 min). The extract significantly ($P < 0.01$) inhibited both the frequency of defaecation as well as the wetness of the faecal droppings of rat (Table 7). Treatment with HEMC in the dose of 200 and 400 mg/kg reduced the weight of defecate as well as reduced the frequency of defecation compared with the control group. The inhibition was 39.15 and 56.8 %, with the dose of 200 and 400 mg/kg respectively. The standard drug loperamide (3 mg/kg) produced an inhibition of 81.19%.

Table 7: Evaluation of anti-diarrhoeal activity of hydroalcoholic extract of *M. coromandelianum* by castor oil induced diarrhoea.

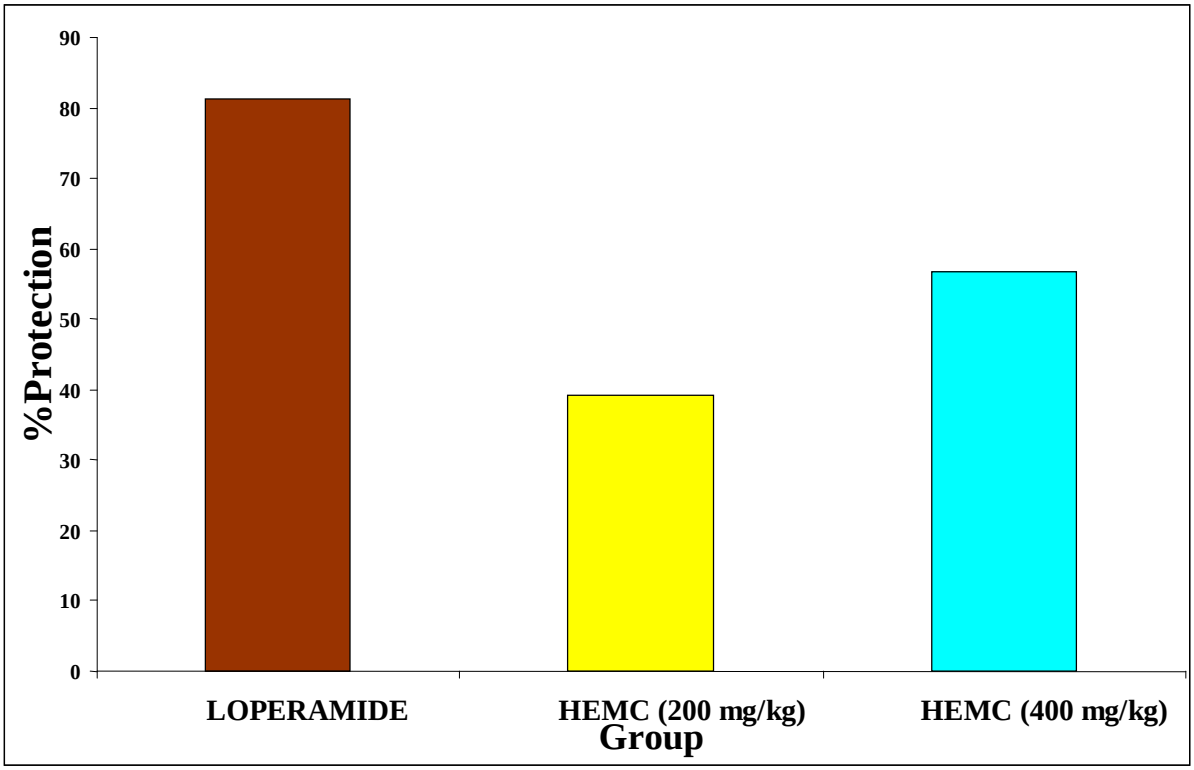
Treatment	MEAN WET DEFECATION	MEAN INCREASE IN WEIGHT OF PAPER (gm)	DELAY IN DEFECATION TIME (min)
Control	9.167 ± 0.87	3.023 ± 0.49	32.167±7.305
Loperamide (3 mg/kg)	1.833 ± 1.05 **	0.533 ± 0.29 **	200.00±22.509**
HEMC (200 mg/kg)	4.667 ± 0.99 **	2.140 ± 0.25^{ns}	70.833±2.845^{ns}
HEMC (400 mg/kg)	3.667 ± 0.80 **	1.407 ± 0.51 *	132.33±29.261**

**=P<0.01= very significant
*=P<0.05= significant
Not significant (ns)=P>0.05
Number of animals (N)=6
Values are expressed as mean±SEM

Table: 8 % Protection against castor oil induced diarrhoea

Treatment	% Protection
Loperamide (3 mg/kg)	81.19
HEMC (200 mg/kg)	39.15
HEMC (400 mg/kg)	56.795

Fig. 13 Evaluation of anti-diarrhoeal activity of hydroalcoholic extract of *M. coromandelianum* by castor oil induced diarrhoea.



5.6 Effect of hydroalcoholic extract of *M. coromandelianum* on intestinal transit

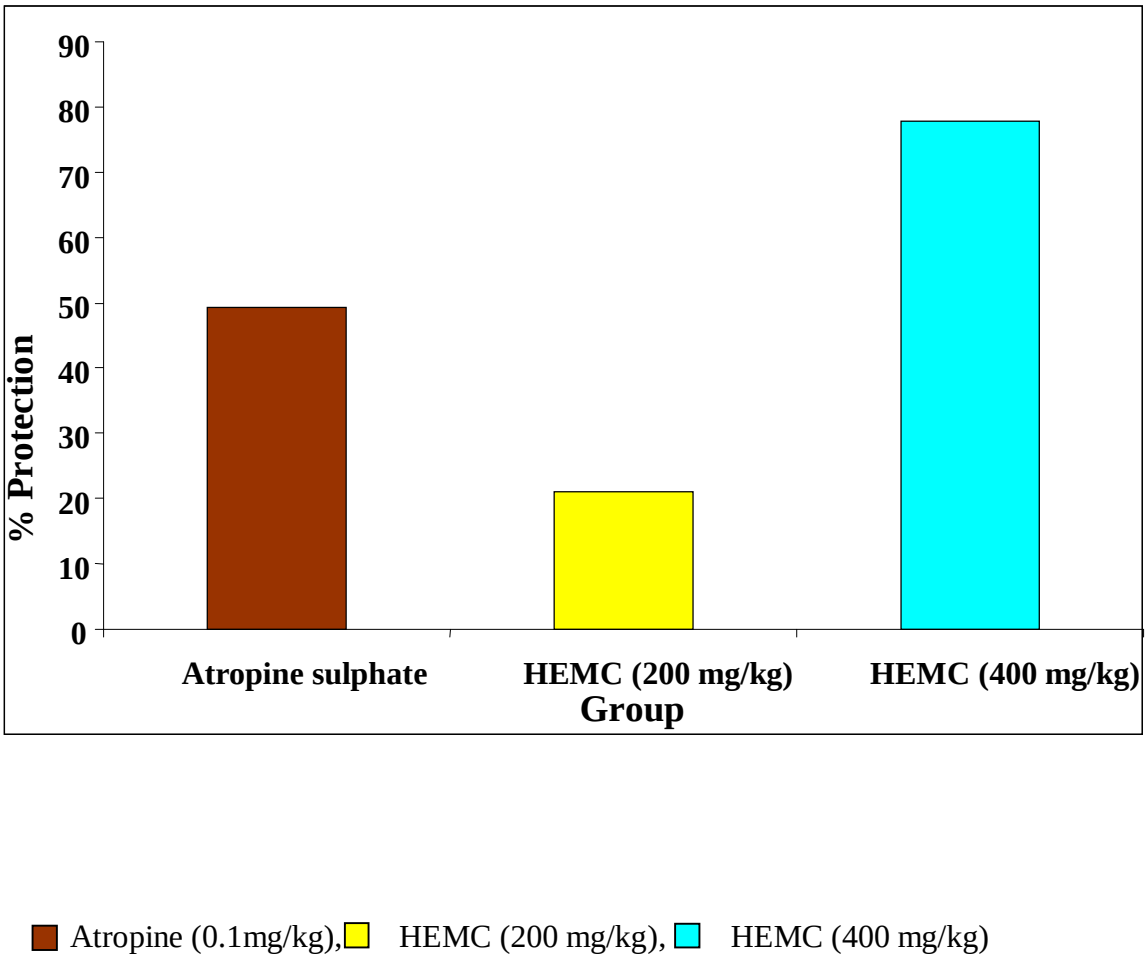
HEMC and atropine sulphate (0.1 mg/kg) decreased the propulsion of the charcoal meal through the gastrointestinal tract when compared with the control (Table 9). Distance traveled by the charcoal meal was reduced to 21.91 and 74.80 % in the HEMC treated groups with the dose of 200 and 400 mg/kg respectively, compared to control group (Table 9). Atropine on the other hand, produced a marked decrease in the propulsive movements and the intestinal length traversed by charcoal meal was 49.87%. These observations were significantly ($P < 0.01$) different from what was seen in the control group. 400 mg/kg dose of the extract exerted greater anti-motility effects than 0.1 mg/kg of atropine (Fig14).

Table 9: Evaluation of anti-diarrhoeal activity of hydroalcoholic extract of *M. coromandelianum* by charcoal meal test.

Treatment	% Movement of charcoal meal
Control	98.440 ± 1.560
Atropine sulphate (0.1 mg/kg)	49.878 ± 8.043**
HEMC (200 mg/kg)	74.802 ± 2.946**
HEMC (400 mg/kg)	21.913 ± 5.188**

**=P<0.01= very significant
Number of animals (N)=6
Values are expressed as mean±SEM

Fig.14 Evaluation of anti-diarrhoeal activity of hydroalcoholic extract of *M. coromandelianum* by Charcoal Meal Test.



5.7 Anti-enteropooling effect of hydroalcoholic extract of *M. coromandelianum*

As shown in Table 10, PGE_2 induced a significant increase in the fluid volume of rat intestine in the control group. The extract inhibited PGE_2-induced enteropooling significantly ($P<0.01$) in rats by both the doses (Table 10). The percentage of reduction of enteropooling was 40.34 and 59.88 with the dose of 200 and 400 mg/kg of HEMC respectively in comparison to control (Fig 5).

Table 10: Evaluation of anti-diarrhoeal activity of hydroalcoholic extract of *M. coromandelianum* by PGE_2 induced enteropooling.

Treatment	Volume of intestinal fluid (ml)
PGE_2 Control	3.240 ± 0.09798
Vehicle Control	3.080 ± 0.1020^{ns}
HEMC (200 mg/kg)	1.933 ± 0.1978**
HEMC (400 mg/kg)	1.300 ± 0.1125**

**=P<0.01= very significant
Not significant (ns)=P>0.05
Number of animals (N)=6
Values are expressed as mean±SEM

Fig.15 Evaluation of anti-diarrhoeal activity of hydroalcoholic extract of *M. coromandelianum* by PGE_2 induced Enteropooling.

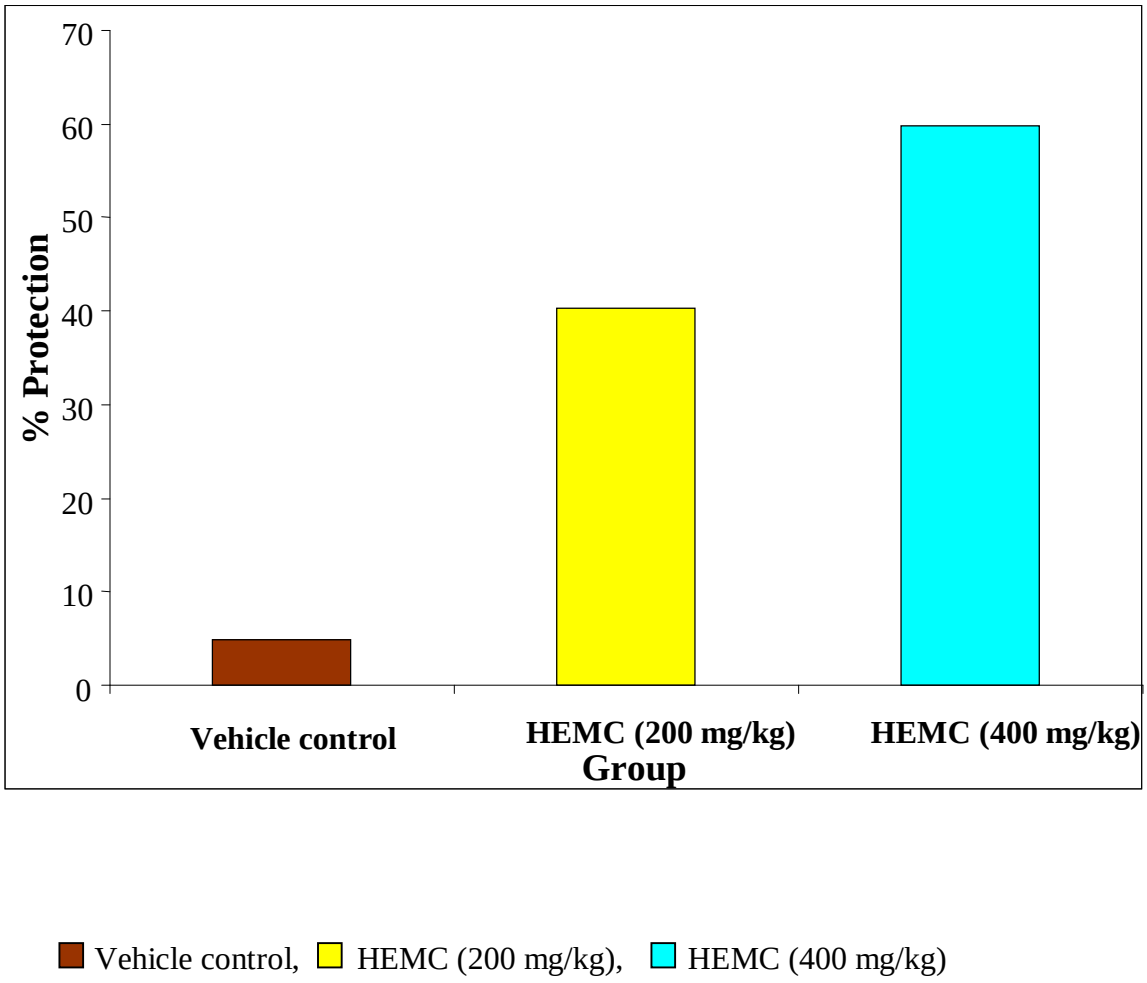
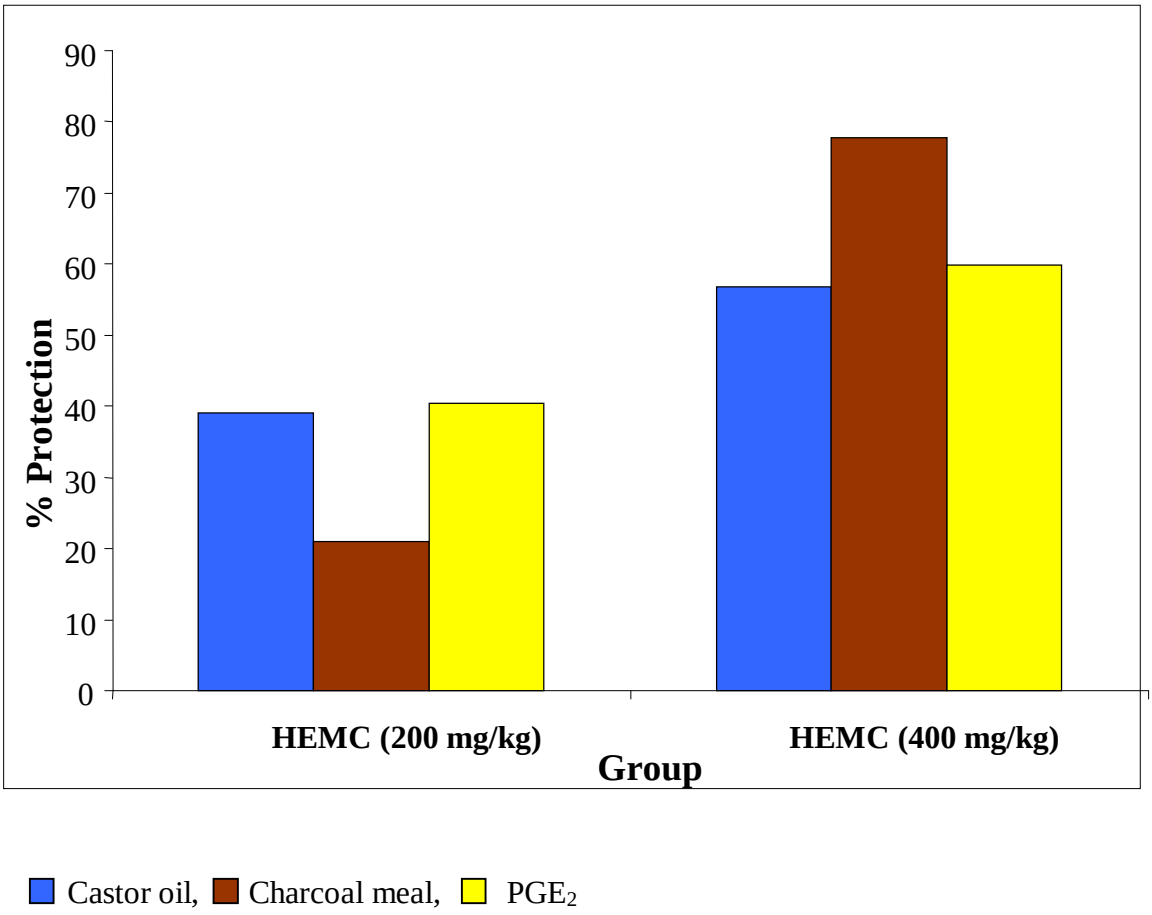


Fig.16 Comparison of protection against diarrhoea.



CHAPTER 6
DISCUSSION

6. DISCUSSION

M. coromandelianum has both peripheral and central analgesic properties. Its central analgesic activity confirmed through tail flick and hot plate method and YSR Reddy et. al. reported its peripheral analgesic property by writhing test[38].

Effect of HEMC on tail flick response provides a confirmation of its central effect since the tail flick is predominantly a spinal reflex and is considered to be selective for centrally acting analgesic compounds, like morphine, pethidine, while peripheral analgesic is known to be inactive on this kind of painful stimulus[92,93]. Nociceptive pathways activated in the tail-flick and hot-plate tests are not the same[94, 95]. Therefore, in different antinociceptive tests the same opioid ligand can elicit different responses, even though the same receptors are involved. This is also supported by our results since the extent of analgesic activity exhibited by tail flick method is greater than the hot plate method. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical (acetic acid) induced nociceptive stimuli. Acetic acid induces hyperalgesic response due to prostaglandin and sympathetic mediators[96,97].

Peripheral analgesic effect of plant's extract may be mediated by prostaglandin inhibition whereas the central analgesic action of the extract probably mediated through inhibition of central pain receptors.

Analgesic effect of extract exhibited after 30 min of its administration. Analgesic effect of extract last after 3 h and shows maximum antinociceptive effect after 1 h of its administration. Both the doses showed analgesic effect. This suggests that chemical constituents by the virtue of them showed pharmacological action has rapid onset of action and of short duration of action.

Carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the anti-edematous effect of natural products[98]. Carrageenan is a polysaccharide known to activate the Hageman factor and to liberate kallikrein from its inactive precursor prekallikrein[99]. Acute inflammation of carrageenan involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, histamine, bradykinins, leucotrienes and serotonine, all of which also cause pain and fever. Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects will normally ameliorate the inflammation and other symptoms.

Development of edema in the paw of the rat after injection of carrageenan is a biphasic event[100]. The initial phase observed during the first hour is attributed to the release of histamine and serotonin[101]. The second phase (2-5 h after injection) of edema is due to the release of prostaglandins, protease and lysosome[101,102]. Based on this, the HEMC has effect on both the phases since it showed effect after 30 min of carrageenan injection. That is the suppression of the first phase may be due to inhibition of the release of early mediators such as histamine and serotonin and also up to 3 h i.e. the second phase. This effect may be by either inhibition of phospholipase A_2 (PLA_2) activity or cyclooxygenase pathway and by blocking the release of vasoactive substances (histamine, serotonin and kinins). Both the doses of extract showed significant protection against edema. It shows maximum effect at 90 min which is comparable to the standard.

The effect could also be related to inhibition of kinins release, which have been implicated as major proinflammatory mediators in addition to histamine and prostaglandins[99,102].

Both the doses of extract showed protection against PGE_2 induced enteropooling, which might be due the inhibition of synthesis of prostaglandins. Finding out of antienteropooling effect of the extract is more relevant because the prevention of enteropooling helps in the inhibition of diarrhea, especially by PGE_2 induced diarrhea. Since, the PGE_2 levels are involved in the onset of diarrhoea in intestinal mucosal cells. Although intraluminally administered PGE_2 is known to induce duodenal and jejunal secretion of water and of electrolytes such as Cl^- and Na^+ [103]. Fluid content is the principal determinant of stool volume and consistency. Net stool fluid content reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along gastrointestinal tract. Neurohumoral mechanisms, pathogens and drugs can alter these processes, resulting in changes in either secretion or absorption of fluid by the intestinal epithelium. Altered motility also contributes in a general way to this process, as the extent of absorption parallels the transit time.

HEMC (200 and 400 mg/kg) and the anti-muscarinic drug, atropine (0.1 mg/kg) decreased the propulsive movement in the charcoal meal study, the atropine being less potent than the aerial part extract of 400 mg/kg. The underlying mechanism appears to be spasmolytic and an anti-enteropooling property by which the extract produced relief in diarrhoea. Tannic acid and tannins are present in many plants and they denature proteins forming protein tannate complex. The complex formed coat over the intestinal mucosa and makes the intestinal mucosa more resistant and reduces secretion[104]. The tannin present in the plant extracts may be responsible for the anti-diarrhoeal activity.

The anti-diarrhoeal effect of the extracts may be related to an inhibition of muscle contractility and motility, as observed by the decrease in intestinal transit by charcoal meal and consequently, in a reduction in intestinal propulsion.

HEMC also inhibited the onset time and severity of diarrhoea induced by castor oil. Castor oil is reported to cause diarrhoea by increasing the volume of intestinal content by prevention of reabsorption of water. Castor oil contains ricinoleic acid which induces irritation and inflammation of the intestinal mucosa, leading prostaglandin release which, in turn, changes in mucosal fluid and electrolyte transport thereby preventing the reabsorption of NaCl and water results in a hypersecretory response and diarrhoea[74,76,105]. The experimental studies in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil[106]. Ricinoleic acid markedly increased the PGE_2 content in the gut lumen and also caused on increase of the net secretion of the water and electrolytes into the small intestine. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea[107].

The diarrhoeal effect of castor oil may be involved NO[83,108], that increase the permeability of the epithelial layer to calcium ions, leading to an increase in intracellular Ca^{2+} and enhancement of calmudin stimulation of NO synthatase activity. NO, in turn, could stimulate intestinal secretion. It is well known that nitric oxide and prostaglandins are crucial mediators contributing to generation of inflammatory response to castor oil. Alternatively, the effect of castor oil may be attributed to disordered motility and hence to an increase in intestinal transit of intraluminal material. In this connection, castor oil could alter coordination of intestinal motility and could promote greater loss of fluid from intestine[109].

The reduction of gastrointestinal motility is one of the mechanisms by which many anti-diarrhoeal agents act[110].

The antidiarrhoeal effect of HEMC is due to reduction of gastrointestinal motility, inhibition of the synthesis of prostaglandin and NO. The HEMC has potential effect on the reduction of gastrointestinal motility than the other effects (Fig.14). The antidiarrhoeal effect of it may also due to the presence of tannins and flavanoids in the extract and probably which are responsible for the above effects.

Flavonoids have been shown to possess a variety of biological activities; they were reported to act in the gastrointestinal tract as either anti-ulcer, anti-spasmodic, anti-secretary or antidiarrhoeal agents.

CHAPTER 7
CONCLUSION

7. CONCLUSION

1. The qualitative phytochemical study reveals the presence of saponins, steroids, Fixed oils, glycosides (anthracene, cardiac), flavonoids and tannins.
2. The present study demonstrated that the hydroalcoholic extract of aerial parts of *Malvastrum coromandelianum* possess analgesic, anti-inflammatory and anti-diarrhoeal property.
3. The anti-inflammatory effect of it could be due to either inhibition of phospholipase A2 (PLA2) activity or cyclooxygenase pathway and by blocking the release of vasoactive substances such as histamine, serotonin and kinins.
4. The central analgesic action of the extract was probably mediated through inhibition of central pain receptors whereas peripheral analgesic effect of plant's extract may be mediated by prostaglandin inhibition.
5. The antidiarrhoeal effect of HEMC is due to reduction of gastrointestinal motility, inhibition of the synthesis of prostaglandin and NO.
6. The HEMC has potential effect on the reduction of gastrointestinal motility than the other effects.
7. The above effects of it may also be due to the presence of tannins and flavanoids in the extract.

Scope for further study

Further study is warranted to isolate, characterize and screen the active principles from the aerial parts of *Malvastrum coromandelianum* that possess analgesic, anti-inflammatory and anti-diarrhoeal effects. And also there is a need to find out the exact mechanism by which the above effects are produced.

CHAPTER 8
SUMMARY

8. SUMMARY

- The present study was designed to investigate the analgesic, anti-inflammatory and anti-diarrhoeal activity of hydroalcholic extract of aerial parts of *Malvastrum coromandelianum*.
- Crude drugs, i.e. aerial parts of *Malvastrum coromandelianum* were collected from the forest of Ghulewadi range and dried under shade. The crude drug was authenticated from Botanical Survey of India, Pune.
- The powder of the drug was packed into a soxhlet apparatus and extracted with petroleum ether ($60\text{-}80^0$) for 18 h. The same marc was successively extracted with chloroform and afterwards with hydroalcohol (30:70) for 18 h.
- Preliminary phytochemical investigation of hydroalcholic extract of aerial parts of *Malvastrum coromandelianum* showed that-
    - Pet. ether extract contains saponins, steroids, fixed oils.
    - Chloroform extract contains saponins, steroids, fixed oils, anthracene and cardiac glycosides.
    - Hydroalcoholic extract contains saponins, steroids, fixed oils, anthracene and cardiac glycosides, flavonoids and tannins.
- Analgesic, anti-inflammatory and anti-diarrhoeal activity of hydroalcholic extract of aerial parts of *Malvastrum coromandelianum* at a dose of 200 and 400 mg/kg was investigated.
- Analgesic activity was assessed by using tail-flick and hot plate method. HEMC produced dose dependent analgesic activity significantly ($P < 0.01$) against tail

flick method. Pretreatment with the extract significantly increased reaction time, compared with control. At a dose of 400 mg/kg of HEMC exhibited analgesic effect to the same degree as morphine 5 mg/kg up to 150 min and at 180 min its effect was significant but lesser than the standard in comparison with control. The peak effect (162.2%) of HEMC 400 mg/kg was shown at 60min which is almost equal to the peak effect (168.39%) of morphine 5 mg/kg. The antinoceptive effect of HEMC by the hot plate method was produced dose dependently on mice. At the dose of 200 mg/kg the effect of HEMC was not significant after 30 min of its administration but its 400 mg/kg dose produced significant ($P<0.01$) effect. The HEMC showed peak effect 120.61% and 155.35% at the dose of 200 mg/kg and 400 mg/kg respectively at 60 min after treatment while morphine was also showed peak effect 197.22% at 60 min in comparison with control.

- Anti-inflammatory activity was assessed by using acute paw oedema induced by carrageenan. The HEMC produced significant ($P < 0.01$) anti-inflammatory activity and the results were comparable to that of diclofenac sodium (100 mg/kg) as a standard anti-inflammatory drug. The maximum percent inhibition was showed 81.73 and 84.57 at the doses 200 and 400 mg/kg of HEMC respectively at 90 min while diclofenac sodium showed 97.38 % at 120 min.
- Anti-diarrhoeal activity was evaluated by using Castor oil induced diarrhoea, charcoal meal test and PGE_2 induced enteropooling.
- The HEMC exhibited pronounced antidiarrhoeal effect in a dose-dependent manner following oral pretreatment on castor oil-induced diarrhoea compared with the control. The extract prolonged the onset time of diarrhoea, 70.83 min and

132.33 min, at the dose of 200 and 400 mg/kg respectively. Although the effect is significant but comparatively it is lesser than the loperamide 3 mg/kg (200 min). The extract significantly ($P < 0.01$) inhibited both the frequency of defecation as well as the wetness of the faecal droppings of rat. The inhibition was 39.15 and 56.8 %, with the dose of 200 and 400 mg/kg respectively.

- Effect of HEMC on intestinal transit was measured by using charcoal meal test. Distance traveled by the charcoal meal was reduced to 21.01 and 77.74 % in the HEMC treated groups with the dose of 200 and 400 mg/kg respectively, compared to control group.
- Anti-enteropooling activity was assessed by using PGE_2 induced diarrhoea. The extract inhibited PGE_2-induced enteropooling significantly ($P<0.01$) in rats at both the doses. The percentage of reduction of enteropooling was 40.34 and 59.88 with the dose of 200 and 400 mg/kg of HEMC respectively.
- The antidiarrhoeal effect of it may also due to the presence of tannins and flavanoids in the extract and probably which are responsible for the above effects.

CHAPTER 9
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9. BIBLIOGRAPHY

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