"FORMULATION AND EVALUATION OF A HERBAL GEL CONTAINING RESINOUS DRUGS FOR ARTHRITIS"

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BY

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I declare that the thesis "Formulation And Evaluation of a Herbal Gel Containing Resinous Drugs for Arthritis", has been prepared by me under the guidance of Dr. Vimal Kumar, HOD, Department of Phytopharmaceuticals and Natural Products, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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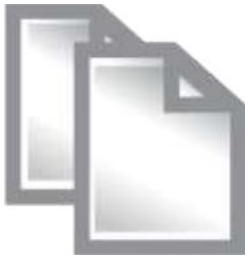

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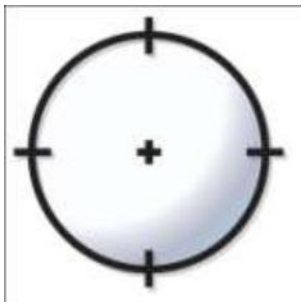

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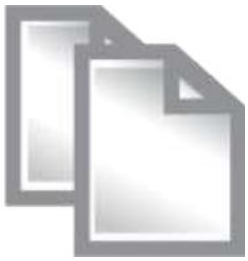
AA	***Arachidonic Acid***
AIA	***Adjuvant Induced Arthritis***
AMLEt	***Aegle marmelos Leaf Extract***
API	***Air Pouch Inflammation***
CACAI	***Candida Albicans Caused Arthritis Inflammation***
CFA	***Complete Freund's Adjuvant***
CIA	***Collagen Induced Arthritis***
CIPO	***Carrageenan Induced Paw Oedema***
CPG	***Cotton Pellet Granuloma***
DMARDs	***Disease Modifying Antirheumatic Drugs***
GM-CSF	***Granulocyte- Macrophage Colony Stimulating factors***
IFN-γ	***Interferon-γ***
IL	***Interleukin***
iNOS	***Inducible Nitric Oxide Synthase***
MAPK	***Mitogen Activated Protein Kinase***
MHC	***Major Histocompatibility Complex***
MPC	***Mononuclear Phagocytes***
NK	***Natural Killer***
NSAIDs	***Non Steroidal Anti-inflammatory Drugs***
PMN	***Poly Morphonuclear leucocytes***
RA	***Rheumatoid Arthritis***
RBL	***Rat Basophillic Leukemia***
TNF	***Tumour Necrosis Factor***



Chapter 1

Abstract

1. ABSTRACT

Rheumatoid arthritis (RA) is an inflammatory disease. It largely affects synovial joints, which are lined with a specialized tissue called synovium. RA typically affects the small joints of the hands and the feet, and usually both sides equally and symmetrically, although any synovial joint can be affected. It is a systemic disease and can affect the whole body, including the heart, lungs and eyes. The overall occurrence of RA is two to four times greater in women than men.

Rheumatoid arthritis in Ayurveda means the imbalance in the basic elements of the human body on which entire body physiology and anatomy is based. As per ancient ayurvedic concept of treating arthritis, the line of treatment is divided into six categories: Drugs having analgesic effects; nervine tonics; anti-inflammatory drugs; anxiolytics; immunomodulators and antioxidants.

Gels are defined as semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Gels are also defined as semisolid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules in the dispersed phase. Physical and/or chemical cross-linking may be involved.

As we have prepared a topical formulation we have excluded nervine tonic from the formulation. We have taken extracts three drugs namely *Zingiber officinalis, Boswellia serrata, Aegle marmelos*. The 5% gel was formulated using hydroalcoholic extract of *Zingiber officinalis*, methanolic extract of *Boswellia serrata* and methnolic extract of *Aegle marmelos* in the ratio of 1:1:1. The gel was optimized using different concentrations of polymer carbopol 934. The herbal drugs used in the formulation have already been proved for their anti-inflammatory activity, analgesic activity, antioxidant activity, immunomodulatory activity and antiarthriric activity. The preliminary studies of the drugs were carried out before the formulation of gel.

The aim of our study were to optimize the gel, to find out the potential of the formulation for the anti-inflammatory, antioxidant and antiarthritic activity and to calculate the amount of standard compound present in the formulation.

The antioxidant activity of the combined extract of the three in the ratio of 1:1:1 and extracts of drugs individually was carried using DPPH free radical scavenging method. The combination shows good antioxidant activity but the antioxidant potential was found to be low in comparison to that of Ginger.

The anti-inflammatory activity of the 5% gel formulation containing three extracts in the ratio (1:1:1) was compared to 5 % gel of individual extracts resulted in significant inhibition of the paw volume to the control group. The reduction in the paw volume was significant with respect to the control group. The percentage inhibition of the formulation was more than the gels containing individual drugs showing synergistic effect between the three drugs.

As the formulation is administered topically, in the adjuvant induced anti-arthritic activity only the paw volume was measured up to the thirteen days after the immunization and the gel formulation has shown significant reduction in the paw volume with respect to that of the control groups.

Each extract of the drug contains many compounds in it. We have estimated only the amount of 6 –Gingerol in the gel according to the standards available in the laboratory through HPTLC. The linearity was observed in concentrations from 200 ng – 1200ng using solvent system hexane: ether (4:6). The plate was scanned at wavelength 254nm. The calibration curve was plotted and the amount of 6-Gingerol present in the gel formulation was calculated.

Chapter 2

Introduction

1. INTRODUCTION

A) Introduction to Rheumatoid Arthritis (RA).

For about a century, rheumatoid arthritis has been recognized as an entity. In recent years, its protean manifestations have received more attention. With the current recognition that there is essentially no organ that may not be affected, it is gradually being referred to as rheumatoid disease with increasing frequency.

RA is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. Rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium, pleura, and sclera, and also nodular lesions, most common in subcutaneous tissue. Although the cause of rheumatoid arthritis is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and RA is considered a systemic autoimmune disease.

About 1% of the world's population is afflicted by rheumatoid arthritis, women are three times more often than men. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated.

It is now not considered as a benign disease. Patients of rheumatoid arthritis experience lower life expectancy than general healthy population. Risk of infections, cardiovascular disease and other co-morbid conditions like depression is also high. Approx 50% patients stop working after 10 years of diagnosis of disease. (The Merck Manuals)

B) Introduction to Gels (David, 2006)

Gels are defined by USP as semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Where the gel mass consists of a network of small discrete particles, the gel is classified

as a two phase system. In two phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes referred to as a magma. Both gels and magmas may be thixotropic, forming semisolid on standing and becoming liquid on agitation.

Single phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. They can be made from synthetic macromolecules or from natural gums. The latter preparations are also called mucilage. Gels can be used to administer drugs topically or into body cavities.

Gels are also defined as semisolid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules in the dispersed phase. Physical and/or chemical cross-linking may be involved. The interlacing and consequential internal friction is responsible for increase in viscosity and the semisolid state.

Some gel systems are clear and others are turbid, since the ingriedients involved may not be completely soluble or insoluble or they may form aggregates, which disperses light. The concentration of the gelling agent is generally less than 10%, usually in the range of 0.5% to 2% range. Gels and magmas are considered as colloidal suspension as both conatin the particle of colloidal dimensions.

The generally accepted size range for a substance “colloidal” is where the particles fall between colloidal 1 nm to 0.5µm. The differences between the colloidal dispersions and true solutions are the particle sizes and optical property where the colloidal dispersions scatter the light where as true solutions do not.

B.1 Gelling Agents:

Several compendia materials function as gelling agents including acacia, alginic acid, bentonte, carbomer, carboxymethylcellulose sodium, cetostearyl alcohol, colloidal silicon oxide, ethylcellulose, gelatin, guargum, hydroxyethyl cellulose, hydroxypropyl

cellulose, magnesium aluminum silicate, maltodextrin, methylcellulose, polyvinyl alcohol, povidone, propylene carbonate, propylene glycol alginate, sodium alginate, sodium starch glycolate, starch, tragacanth, and xanthum gum.

Carbomer resins are high molecular weight, acrylic acid based polymers. The pH of 0.5% and 1.0% aqueous dispersions are 2.7-3.5 and 2.5-3.0 respectively. There are many carbomer resins, with viscosity ranges available from 0 to 80,000 cPs., depending upon the pH to which it is neutralized. In addition to thickening, suspending and emulsifying in both oral and topical formulations, carbomers are also used to provide sustained release properties in both the stomach and the intestinal tract for commercial products. Alcohol is often added to carbomer gel to decrease their viscosity. Carbomer gel viscosity is also dependent upon the presence of electrolytes and the pH. Generally, a rubbery mass forms if greater than 3 % electrolytes are added. Carbomer preparations are primarily used in aqueous systems, although other liquids can be used. In water, a single particle of carbomer will wet very rapidly but like many other powders, carbomer polymer tends to form clumps of particles when haphazardly dispersed in polar solvents. Rapid dispersion of carbomers can be achieved by adding the powder very slowly into vortex of the liquid that is very rapidly stirred. A neutralizer is added to thicken the gel after the carbomer is dispersed. Sodium hydroxide or potassium hydroxide can be used in carbomer dispersions containing less than 20 % alcohol. Triethanolamine will neutralize carbomer resins containing up to 50% alcohol.

B.2 Single Phase Gels

Single phase gels are used more frequently in pharmacy for several reasons: semisolid state, high degree of clarity, ease of application and ease of removal and use. The gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments.

Gel formulations include ophthalmic preparations of pilocarpine and carbachol; topical preparations for burn therapy, anti-inflammatory treatment, musculoskeletal disorders, and acne; peptic ulcer treatment with sucralfate gel; and bronchoscopy using lidocaine.

Gels may be used as lubricants for catheters and bases for patch testing, and sodium chloride gels are used for electrocardiography.

C) Paw Edema in Rats (Vogel, 2002)

Among many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, Aerosil®, sulfated polysaccharides like carrageenan or naphthoylheparamine. The effect can be measured in several ways. The hind limb can be dissected at the talocrural joint and weighed. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. Methods have been described to measure the paw volume by simple and less accurate and by more sophisticated electronically devised methods. The value of the assessment is less dependent on the apparatus but much more on the irritant being chosen. Some irritants induce only a short lasting inflammation whereas other irritants cause the paw edema to continue over more than 24 h. Carrageenan is linear sulfated polysaccharides that are extracted from red seaweeds. There are three main commercial classes of carrageenan:

- **Kappa** forms strong, rigid gels in the presence of potassium ions. It reacts with dairy proteins obtained from *Eucheuma cottonii*.
- **Iota** forms soft gels in the presence of calcium ions. It is produced mainly from *Eucheuma spinosum*.
- **Lambda** does not gel, and is used to thicken dairy products, obtained from *Gigartina.*

The primary differences that influence the properties of kappa, iota, and lambda carrageenan are the number and position of the ester sulfate groups on the repeating galactose units. Higher levels of ester sulfate lower the solubility temperature of the

carrageenan and produce lower strength gels, or contribute to gel inhibition. Animal models of inflammation uses lambda form (dilute lambda carrageenan solution 1–2%) injected subcutaneously causes swelling and pain.

C.1 Inflammation Inducing Agents

Agents can be used as irritants to induce paw edema in rats or mice are:

0.05 ml undiluted fresh egg white, 0.1 ml of 1% ovalbumin solution, 0.1 ml of 1% formalin, 0.1 ml of 1% carrageenan solution plus 100 ng PGE2 or PGI2, 0.1 ml of 1 to 3% dextran solution, 0.1 ml of 2.5% brewer's yeast powder suspension, 0.1 ml of 0.5% ß-naphthoylheparamine solution, 0.1 ml of 0.1% trypsin solution, 0.1 ml of 0.1% collagenase solution, 0.1 ml of 0.1% solution of collagenase from *Clostridium histolyticum,* 0.1 ml of solution of 100 IU hyaluronidase, 0.1 ml of complete Freund's adjuvant, 0.05 ml of 0.02% serotonin solution, 0.1 ml of 0.005% bradykinin solution, 0.1 ml of 0.1 mg/ml prostaglandin E2, 0.1 ml of 2.0 μg/ml prostaglandin E2, 0.1 ml of 1% concanavalin A solution, 0.1 ml of 2.5% suspension of Aerosil®, 0.1 ml of 5% suspension of kaolin, 0.05 ml of bentonite gel, 0.1 ml of nystatin 15 000 units, 0.1 ml of 1% phytohaemagglutinin-P solution, 0.01 ml of 0.5% adriamycin, 0.1 ml of 0.001–0.1% solutions of various phospholipases A2, 0.1 ml of 0.1% Zymosan solution, 0.1 ml of 0.05% anti-IgG solution, 0.1 ml of 2.5% mustard powder suspension, 0.1 ml of solution containing 1 unit of cobra venom factor, 0.05 ml of 0.02–0.2% sonic extract from *Porphyromonas Gingivalis,* 0.1 ml of 0.25% suspension of papaya.

D) Adjuvant Induced Arthritis in Rats

Adjuvant Induced Arthritis (AIA) is an induced form of chronic arthritis. Strains of rats have a varying genetic susceptibility to AIA, whereas mice generally are not susceptible. AA is most easily induced with mycobacteria (heat killed *Myobacterium tubercolusis*) suspended in oil, although in some strains of rats it can be induced with oily adjuvants in the absence of mycobacteria. The disease is a T-cell–mediated autoimmune arthritis that is frequently used to study immunological aspects of rheumatoid arthritis and other arthritic or inflammatory diseases in humans. Furthermore, it is used as a model for developing and testing anti-inflammatory drugs. There are no particularly well-defined

autoantigens in AIA; in this respect, the model resembles spontaneous arthritic diseases in humans. In all susceptible rat strains, the inflammatory process of AIA is self remitting, although usually the disease is severe and leads to permanent joint malformations, including ankylosis. (Eden et *al.,* 1996)

AIA was initially observed by accident when Complete Freund's Adjuvant (CFA) was used for immunization. Since then, induced AA has been used extensively as a model for rheumatoid arthritis, although some histopathological differences exist. By its nature, where the disease is induced by a bacteria-containing substance, AIA can also serve as a model for reactive arthritis. Some of the additional clinical features of reactive arthritis are also seen in AIA: e.g., iridocyclitis, nodular skin lesions, genitourinary lesions, and diarrhea. Besides this, the model features some aspects of rheumatic fever: e.g., ulcerative colitis and arcoidosis. (Biliau and Matthys, 2001)

D.1 Mechanisms of Action

Freund had suggested three categories of action mechanisms:

1) Prolong the presence of antigens at the site of injection,

2) More effective "transport of the antigens to the lymphatic system and to the lungs, where the adjuvant promotes the accumulation of cells concerned with the immune response," and

3) Other mechanisms that should remain unidentified, because their clarification would require knowledge about "how antibodies are formed and how sensitization develops". (Freund, 1956)

Primary target cells for the adjuvant components are mononuclear phagoctyes (MPC) and direct cells (DCs), which can produce tumour necrosis factor α (TNF-α), interleukin 12 (IL-12), and IL-6. Early interferon-γ (IFN-γ) may come from natural killer (NK) cells, which may become involved as soon as IL-12 appears on the scene but may also be triggered more directly through a pathway involving their activating receptor, NKGD2, which recognizes major histocompatibility complex (MHC-I) like antigens induced on several cells by stress signals (Cerwenka and Lanier, 2001). IL-12 and IFN-γ form a

positive feedback loop that potentiates deviation toward Th1-type responsiveness of activated CD4+ T cells. Production of TNF-α can be presumed to play a role as inducer of other cytokines (such as IL-6) and chemokines. IL-6 may play a role as stimulator of autoantibody production and activator of T-lymphocytes. In the later phases, as disease becomes overt, long-lived CFA components may continue to stimulate cytokine production, but activated lymphocytes conceivably now play an increasingly important part. One effect of prolonged cytokine production, in particular of IL-12 and IL-6, is to generate a myelopoietic response, which at least in some models, constitutes a disease-promoting factor (Matthys *et al.,* 2000). IFN-γ produced in this phase can still act as an up-regulator of MPC-like effector cells and thus potentially play a disease promoting role. However, via another pathway, IFN-γ can act against disease by counteracting the myelopoietic effect of CFA.

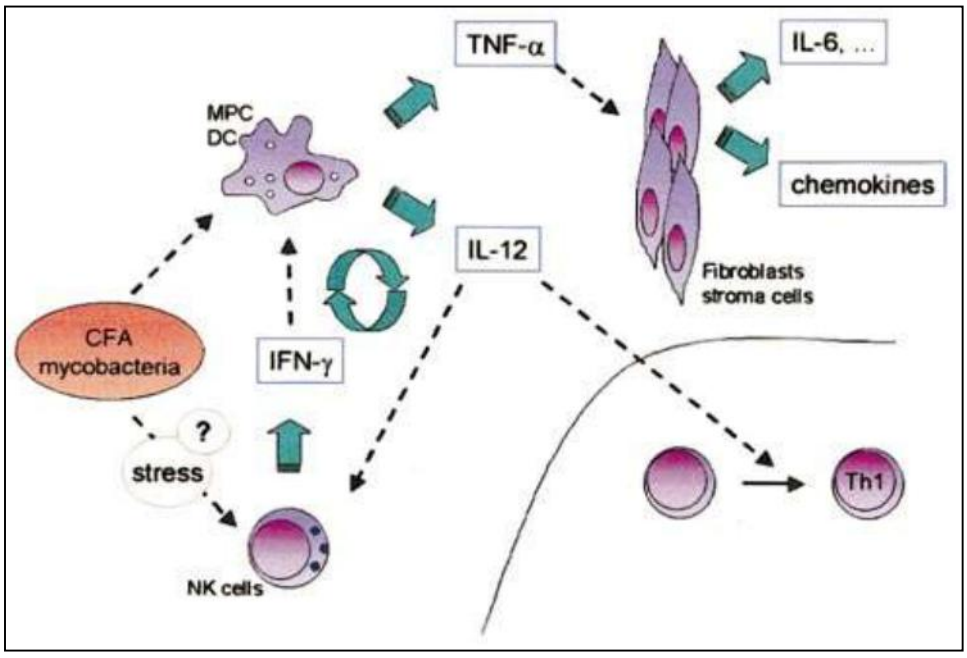


Fig 2.1 Chemokine induction

AIA is a rather aggressive, monophasic, and self-remitting form of arthritis. This means that the time period that can be used for measuring effects of drugs or other agents to modulate disease is limited. Therefore, in some cases it may be desirable to start the

modulation before the disease becomes clinically apparent, e.g., between days 7 and 10 after immunization.

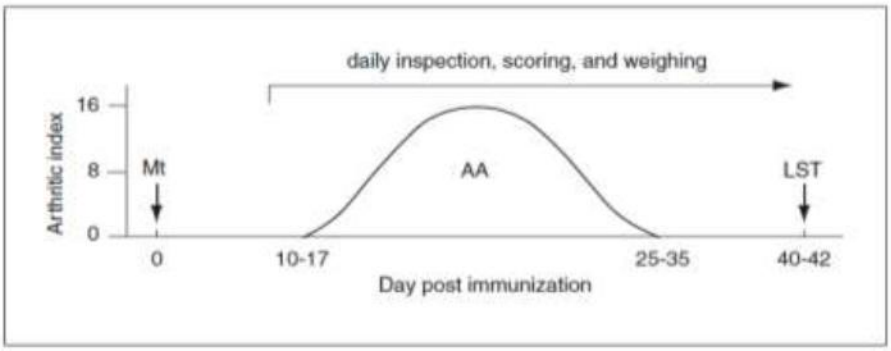


Fig 2.2 Time line for key events in the development of AIA. Mt, injection of Mycobactimulatioerium tuberculosis; LST, lymphocyte stimulatiton test, a proliferative assay of T cell response.

E) Role of Antioxidants in RA (Mahajan and Tandon, 2004)

Free radical can be defined as a chemical species, an atom or a molecule that has one or more unpaired electrons in its valance shell and is capable of existing independently. Free radical attacks the nearest stable molecule "stealing" its electron. When the attacked molecule looses its electron, it becomes a free radical itself, beginning a chain reaction cascade resulting in disruption of a living cell.

Rheumatoid arthritis is a systemic disease characterized by progressive, erosive, and chronic polyarthritis. Cellular proliferation of the synoviocytes and neo-angiogenesis leads to formation of pannus which destroys the articular cartilage and the bone. Recent studies provide evidences for the involvement of free radical/ Reactive Oxygen Species in the pathogenesis of rheumatoid arthritis.

A study by Karatas indicated that increased oxidative stress and/or defective antioxidant status contribute to the pathology of rheumatoid arthritis. The study showed raised levels of Malondialdehyde and low levels of endogenous antioxidants in patients of rheumatoid arthritis. Plasma catalase had also been reported to be significantly lower in patients with RA. Another study reported impaired glutathione reductase activity in synovial fluid in rheumatoid arthritis. In active RA and juvenile idiopathic arthritis, increased oxidative stress and decreased levels of antioxidants have been reported.

Chapter 3

Literature Review

3. LITERATURE REVIEW

A) Rheumatoid Arthritis:

A.1 Introduction: Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. Rheumatoid arthritis can also cause inflammation of the tissue around the joints, as well as in other organs in the body. It causes pain, swelling, stiffness and loss of function in joints. It can affect any joint but is common in the wrist and fingers. More women than men get rheumatoid arthritis. Since it can affect multiple other organs of the body, rheumatoid arthritis is referred to as a systemic illness and is sometimes called rheumatoid disease.

While rheumatoid arthritis is a chronic illness, meaning it can last for years, patients may experience long periods without symptoms. However, rheumatoid arthritis is typically a progressive illness that has the potential to cause joint destruction and functional disability. RA decreases life expectancy by 3 to 7 yr, with heart disease, infection, and gastrointestinal bleeding accounting for most excess mortality; drug treatment, cancer, as well as the underlying disease may be responsible. (Newman and Matzko, 2007)

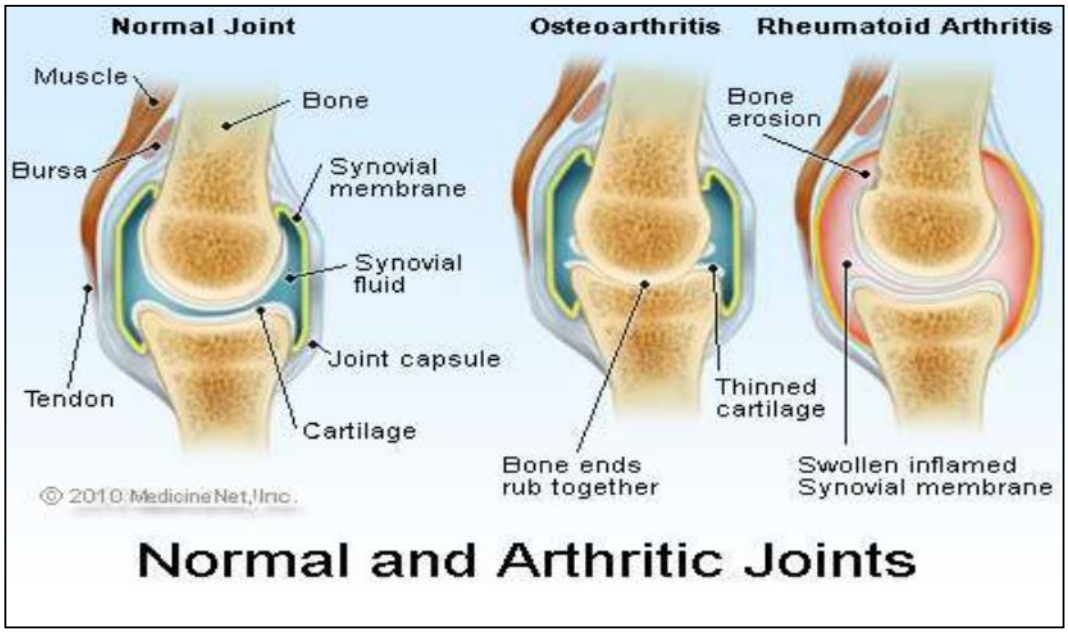


Fig 3.1 Normal and Arthritic Joints

A.2 Causes: The cause of rheumatoid arthritis is unknown. Even though infectious agents such as viruses, bacteria, and fungi have long been suspected, none has been proven as the cause. It is believed that the tendency to develop rheumatoid arthritis

may be genetically inherited. It is also suspected that certain infections or factors in the environment might trigger the activation of the immune system in susceptible individuals. This misdirected immune system then attacks the body's own tissues. This leads to inflammation in the joints and sometimes in various organs of the body, such as the lungs or eyes.

It is not known what triggers the onset of rheumatoid arthritis. Regardless of the exact trigger, the result is an immune system that is geared up to promote inflammation in the joints and occasionally other tissues of the body. Immune cells, called lymphocytes, are activated and chemical messengers (cytokines, such as tumor necrosis factor/TNF, interleukin-1/IL-1, and interleukin-6/IL-6) are expressed in the inflamed areas.

Environmental factors also seem to play some role in causing rheumatoid arthritis. For example, scientists have reported that smoking tobacco increases the risk of developing rheumatoid arthritis. (Medicinenet.com)

A.3 Symptoms and Signs

Onset is usually insidious, often beginning with systemic and joint symptoms. Systemic symptoms include early morning stiffness of affected joints, generalized afternoon fatigue and malaise, anorexia, generalized weakness, and occasionally low-grade fever. Joint symptoms include pain, swelling, and stiffness. The disease progresses most rapidly during the first 6 yr, particularly the first year; 80% of patients develop some permanent joint abnormalities within 10 yr. The course is unpredictable in individual patients.

Joint symptoms are characteristically symmetric. Typically, stiffness lasts > 60 min on rising in the morning but may occur after any prolonged inactivity. Involved joints become tender, with erythema, warmth, swelling, and limitation of motion. The joints involved include the following:

- Wrists and the index and middle metacarpophalangeal joints (most commonly involved)
- Proximal interphalangeal joints
- Metatarsophalangeal joints

- Shoulders, Elbows, Hips, Knees, Ankles (Medicinenet.com)

A.4 Pathophysiology of Rheumatoid Arthritis

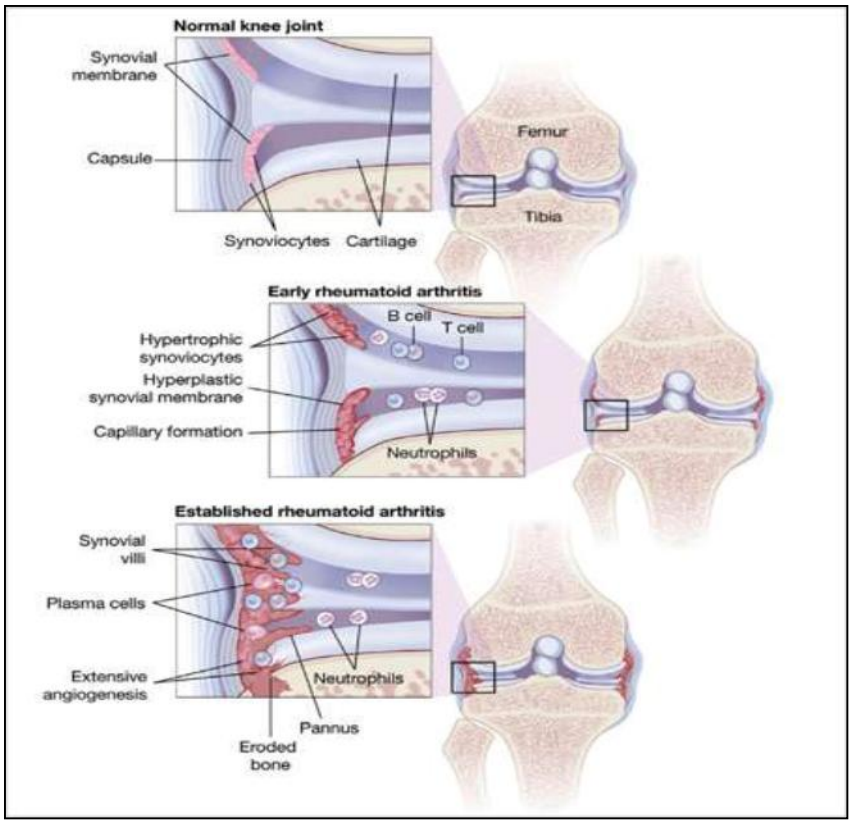


Fig 3.2 Pathophysiology of RA

Prominent immunologic abnormalities include immune complexes produced by synovial lining cells and in inflamed blood vessels. Plasma cells produce antibodies that contribute to these complexes, but destructive arthritis can occur in the absence of rheumatoid factor. Macrophages also migrate to diseased synovium in early disease; increased macrophage-derived lining cells are prominent along with vessel inflammation. Lymphocytes that infiltrate the synovial tissue are primarily CD4+ T cells. Macrophages and lymphocytes produce pro-inflammatory cytokines and chemokines (eg., [TNF], granulocyte-macrophage colony-stimulating factor (GM-CSF), various ILs, IF-γ) in the synovium. Release of inflammatory mediators probably contributes to the systemic and joint manifestations of RA.

In chronically affected joints, the normally thin synovium thickens and develops many villous folds. The synovial lining cells produce various materials, including collagenase and stromelysin, which contribute to cartilage destruction, and IL-1 and

TNF-α, which stimulate cartilage destruction, osteoclast-mediated bone absorption, synovial inflammation, and prostaglandins. Fibrin deposition, fibrosis, and necrosis are also present. Hyperplastic synovial tissue releases these inflammatory mediators, which erode cartilage, subchondral bone, articular capsule, and ligaments. Polymorphonuclear leucocytes on average make up about 60% of WBCs in the synovial fluid. (The Merckmanuals Online Medical Library)

IL-1 is a key mediator of synovial inflammation and pannus formation. It is a pivotal cytokine involved in the pathophysiology of RA. IL-1 is a proinflammatory cytokine that amplifies and perpetuates the disease process in RA. In RA, there are increased amounts of IL-1 in the synovium. In RA patients, IL-1 plasma levels correlate with disease activity. RA patients with erosive disease have higher synovial and circulating levels of IL-1 than patients without erosions.

IL-1 up regulates the production of prostaglandins and other proinflammatory mediators and thereby accounts for pain, swelling and tenderness typically seen in rheumatoid joint inflammation. IL-1 is a key mediator of synovial inflammation and pannus formation. It is a pivotal cytokine mediating destruction of bone and cartilage in RA, and also is believed to impair bone cartilage repair. (Rheumatology Information Service Europe).

A.5 Treatment

RA usually requires lifelong treatment, including medications, physical therapy, exercise, education, and possibly surgery. Early, aggressive treatment for RA can delay joint destruction.

A.5.1 Medication:

Disease modifying antirheumatic drugs (DMARDs): These drugs are the current standard of care for RA, in addition to rest, strengthening exercises, and anti-inflammatory drugs.

Anti-inflammatory medications: These include aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen.

Corticosteroids: These medications work very well to reduce joint swelling and inflammation. Because of long-term side effects, corticosteroids should be taken only for a short time and in low doses when possible.

A.5.2 Surgery

Occasionally, surgery is needed to correct severely affected joints. Surgeries can relieve joint pain and deformities. The first surgical treatment may be a synovectomy, which is the removal of the joint lining (synovium). At some point, total joint replacement is needed.

A.5.3 Physical Therapy

Range-of-motion exercises and exercise programs, joint protection techniques, heat and cold treatments, and splints or orthotic devices to support and align joints may be very helpful. Frequent rest periods between activities, as well as 8 to 10 hours of sleep per night, are recommended. (The Merck Manuals Online Library)

A.6 Herbal therapy for Rheumatoid Arthritis

Rheumatoid arthritis in ayurveda means the imbalance in the basic elements of the humn body on which entire body physiology and anatomy is based. Therefore, improving the function of 'AGNI' and detoxifying 'AMA' becomes a pivotal basis for the initial treatment. Hence ayurvedic practioner or vaidya begins his prescription with simple remedies like Shunti (Ginger) and Eranda (*Ricinus communis* oil) etc., which can perform the task of maintain a proportion and equlibirium between AMA and KAPHA in the body. Patients are strictly advised to ignore the factors spoiling one f the another basic substances of the body 'PITTA' like improper eating, heavy diet and physical and mental stress. As per ancient ayurvedic concept of treating Arthritis, the line of treatment is divided into the following six categories:

- Drugs having analgesic effects
- Nervine tonic
- Anti-inflammatory drugs
- Anxiolytics
- Immunomodulators and Antioxidants (Khan, 2008)

B) *Zingiber officinalis* Roscoe.

A herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2-3 cm broad with sheathing bases, the blade gradually tapering to a point. Inflorescence solitary, lateral radical pedunculate oblongcylindrical spikes. Flowers are rare, rather small, calyx superior, gamosepalous, three toothed, open splitting on one side, corolla of three sub equal oblong to lanceolate connate greenish segments. The plant is widely cultivated all over India, Bangladesh, Taiwan, Jamaica and Nigeria.

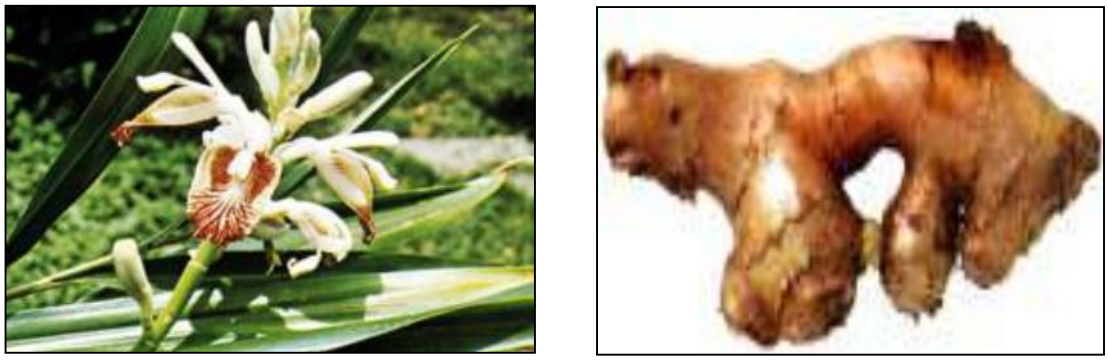


Fig 3.3 Zingiber officinalis: plant and rhizome

B.1 Taxonomical Classification

Kingdom: Plantae

Division: Angiospermae

Class: Monocotyledoneae

Order: Scitaminaea

Family: Zingiberaceae

Genus: *Zingiber*

Species: *officinalis* Roscoe.

B.2 Synonyms

English: Ginger, **Hindi:** Adarak, Sonth (dry root), **Sanskrit:** Adrakam, **Gujarati:** Sunt, **Kannad:** Hasisunti, **Marathi:** Nisam, **Tamil:** Inji, Shukku, **Telugu:** Allaama , Allamu, **Urdu:** Adi. (Warrier *et al.,* 1996)

B.3 Macroscopical Characteristics of Rhizome

B.3.1 Colour & Appearance: Yellowish Brown or light brown. Scraped rhizome with buff external surface showing longitudinal striations and occasional loose fibers, outer surface dark brown and more or less covered with cork which shows conspicuous, narrow, longitudinal and transverse ridges.

B.3.2 Odour: Aromatic

B.3.3 Taste: Pungent. (Mukherjee, 2008)

B.4 Microscopical Characteristics of Rhizome: Smoothed transversely cut surface exhibiting a narrow cortex separated by an endodermis from a much wider stele, numerous widely scattered fibrovascular bundles, abundant scattered oleoresin cells with yellow contents. Starch abundant in the thin-walled ground tissue, as flattened, ovate to subrectangular, transversely straited, simple granules, each with the hilum in a projection towards one end. Pigments cells with dark reddish brown contents occurring either singly in the ground tissue or in axial rows accompanying the vascular bundles. Vessels with spiral or reticulate thickening in the scattered vascular bundles are found. Irregularly shaped thin-walled fibers with delicate, transverse septa, yielding only slightly the reaction characteristic of lignin. sclereids and calcium oxalate crystals absent. (Wallis, 2005)

B.5 Chemical Constituents:

The odour of ginger depends mainly on its volatile oil, the yield of which varies from 1% to 3%. Over 50 components of the oil have been characterized and these are mainly **monoterpenoids**: β-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol and **sesquiterpenoids** : α-zingiberene (30–70%), β-sesquiphellandrene (15–20%), β-bisabolene (10–15%), (*E*-*E*)-α-farnesene, *ar*-curcumene, zingiberol.(Evans 2002).

The pungency of fresh ginger is due primarily to the **gingerols**, which are a homologous series of phenols. The most abundant is [6]-gingerol, although smaller

quantities of other gingerols with different chain lengths, [4]-, [7]-, [8]-, and [10]-gingerol are also present. The pungency of dry ginger mainly results from shogaols [4]-, [6]-, [8]-, [10]- and [12]-shogaol (Jolad *et al.,* 2004)

Paradols are 5-deoxygingerols. [6]-Paradol, along with [7]-, [8]-, [9]-, [10]-, [11]-, and [13]-paradols were detected in the fresh ginger methyl [8]-paradol, methyl [6]-isogingerol and [6]-isoshogaol.

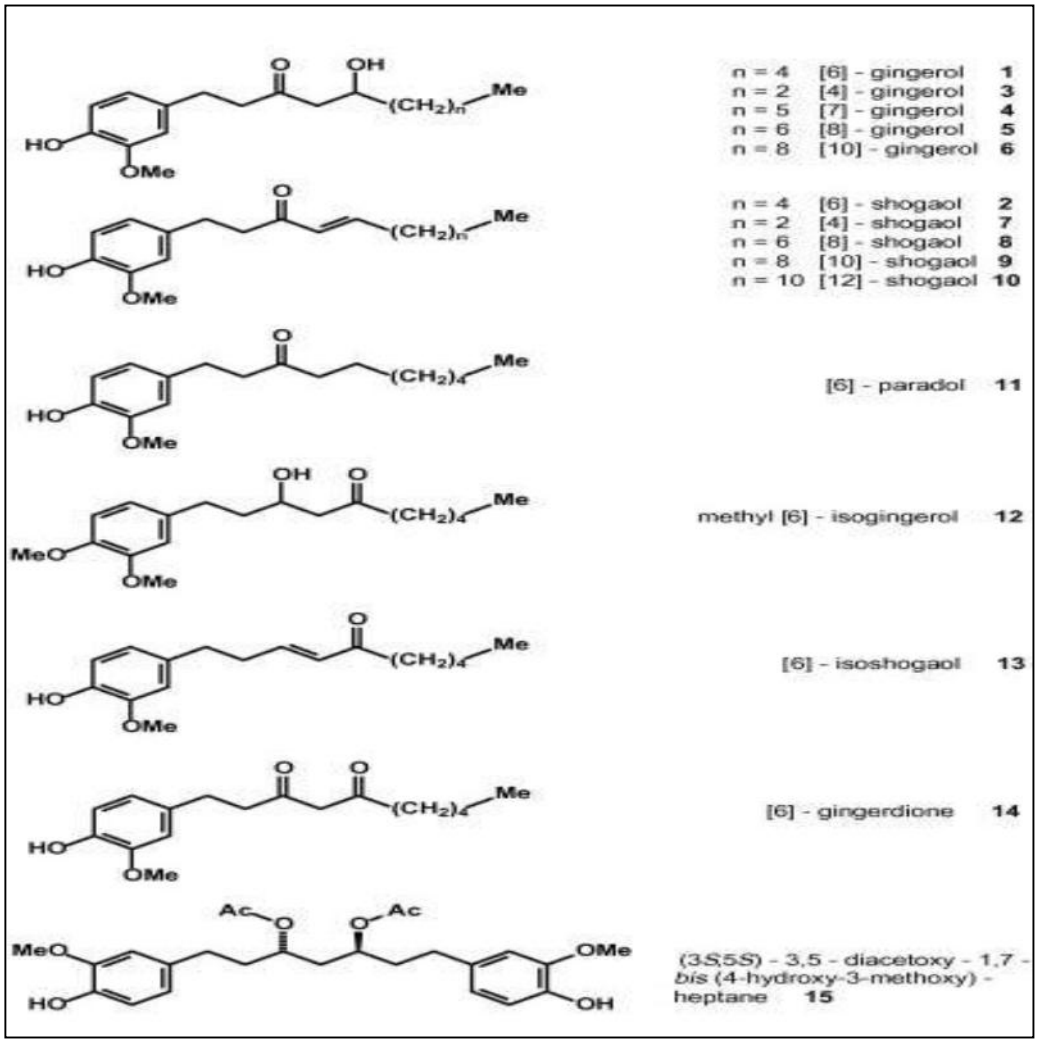


Fig 3.4 Chemical constituents of *Z. officinalis*

B.6 Traditional Uses: Ginger is carminative, pungent, stimulant, used widely for indigestion, stomachache, malaria and fevers. It is said to be used for abdominal pain, anorexia, arthritis, atonic dyspepsia, bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, cystic fibrosis, diarrhoea, difficulty in breathing, dropsy, fever, flatulent, indigestion, disorders of gallbladder, hyperacidity, hypercholesterolemia, hyperglycemia,

indigestion, morning sickness, nausea, rheumatism, sore throat, throat ache, stomach ache and vomiting. (Warrier *et al.,* 1996)

B.7 Pharmacological Properties:

Kiuchi et al. in 1982 reported for the first time that ginger has anti-inflammatory actions, as evidenced by its inhibitory effects on prostaglandins synthesis. It was studied that ethanolic extract reduced carrageenan-induced paw swelling and yeast-induced fever, but was ineffective in suppressing the writhing induced by intraperitoneal acetic acid (Mascolo *et al.,* 1989)

Kiuchi et al., in 1992 had further shown that gingerols are very active in inhibiting both prostaglandins and leukotrienes in RBL-1 (Rat Basophillic Leukaemeia) cells, and that gingerols with long alkyl side chains are more potent inhibitors of leukotrienes synthesis than of prostaglandins synthesis.

Thomson et al. in 2002 had confirmed the inhibitory action of ginger on prostaglandins when they reported that either oral or intraperitoneal administration of a raw aqueous extract of ginger (500 mg/kg) given to rats daily for 4 weeks was effective in significantly reducing serum prostaglandin-E2.

Penna et al., in 2003 investigate the effects of the crude hydroalcoholic extract of ginger rhizomes on the models of rat paw and skin edema. The carrageenan-, compound 48/80- or serotonin-induced rat paw edema were inhibited significantly by the i.p. administration of alcoholic ginger extract at the dose of 186mg/kg body weight. Ginger extract was also effective in inhibiting 48/80-induced rat skin edema at doses of 0.6 and 1.8 mg/site.

Gingerols and their derivatives, especially [8]-paradol, have been reported to be more potent anti-platelet and cyclo-oxygenase-1 (COX-1) inhibitors than aspirin, when tested in vitro by the Chrono Log whole blood platelet aggregometer. (Nurtjahja *et al.,* 2003). It was proposed that the carbonyl functional group at C3 found in paradol and in the diarylheptanoid series may contribute to their potent anti-platelet activity and inhibition of COX-1. Inhibition of the arachidonic acid (AA) metabolism cascade

via the COX-1/thromboxane synthase system by these phenolic compounds may underline the mechanism of their action.

In an in vitro study, the methanolic extract of Z. officinale suppresses inflammation due to arthritis through suppression of pro-inflammatory cytokines and chemokines produced by synoviocytes, chondrocytes, and leukocytes. (Ali *et al*. 2008)

Tripathi et al. in 2003 tested the hypothesis that whole ginger extract has a global inhibitory effect on macrophages function in vitro and that this accounts for its reputed anti-inflammatory effect in vivo. They also hypothesized that the active constituent in ginger, [6]-gingerol, is an effective anti-inflammatory substance because of its inhibition of macrophage activation, more specifically by its inhibition of pro-inflammatory cytokines and antigen presentation by lipopolysaccharide-activated macrophages.

More recently, it has been shown that ginger is effective against cytokines synthesized and secreted at sites of inflammation. Ginger was found to modulate some biochemical pathways activated in chronic inflammation (Grzanna *et al.,* 2005). It was found to inhibit the induction of several genes involved in the inflammatory response, and some of these genes encode cytokines, chemokines and the inducible enzyme cyclo-oxygenase-2 (COX-2).

It was concluded that [6]-gingerol selectively inhibits production of pro-inflammatory cytokines from macrophages, but does not affect the antigen presenting cells (APC) function. Therefore, [6]-gingerol acts as an anti-inflammatory compound that may be useful to treat inflammation without interfering with the antigen presenting function of macrophages. (Young *et al.,* 2005)

It has been examined the effect of a stable [6]-gingerol metabolite, RAC-[6]-dihydroparadol ([6]-DHP) and a closely related gingerol analog, RAC-2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one [a capsaicin/gingerol (capsarol) analog on nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) activity and protein expression levels in a murine macrophage cell line. (Aktan *et al.,* 2006) It has been found that both suppressed NO production in murine macrophages by partially

inhibiting iNOS enzymatic activity and reducing iNOS protein production, via attenuation of NF-kappa B-mediated iNOS gene expression, providing a possible mechanism of action for the anti-inflammatory activity reported for this class of compounds. (Kim *et al.,* 2005)

It is established that neither ginger nor its constituents produce the gastrointestinal adverse effects that are usually produced by the conventional NSAIDs as a result of prostaglandin inhibition (Konturek *et al.,* 2005).

Dedov et al., in 2002 suggested that gingerols act as vanilloid receptor (VR) agonists. The VR1 receptor has been shown to integrate chemical and thermal nociceptive stimuli (Ma and Quirion, 2007). Therefore, direct activation/deactivation of the VR1 receptor at the site where pain is generated during inflammation and other painful conditions provides a new strategy for the development of a new class of peripheral analgesics devoid of the well characterized side effects of currently available analgesics and anti-inflammatory drugs.

It is showed through the Ultrafiltration Liquid Chromatography Mass Spectrometry of a chloroform partition of a methanol extract of ginger roots for COX-2 ligands, and 10-gingerol, 12-gingerol, 8-shogaol, 10-shogaol, 6-gingerdione, 8-gingerdione, 10-gingerdione, 6-dehydro-10-gingerol, 6-paradol, and 8-paradol bound to the enzyme active site. Purified 10-gingerol, 8-shogaol and 10-shogaol inhibited COX-2 with IC50 values of 32 μM, 17.5 μM and7.5 μM, respectively. 10-gingerol, 8-shogaol and 10-shogaol inhibit COX-2 but not COX-1. (Richard *et al.,* 2010)

Ginger has been reported that treatment with methanolic extract of dried rhizomes of ginger produced a significant reduction in fructose-induced elevation of lipid levels, bodyweight, hyperglycemia and hyperinsulinemia. (Kadnur and Goyal, 2005).

Thomsan et al in 2002 reported the significant reduction of thromboxaneB_2 when aqueous extract of ginger was given at dose of 500 mg/kg orally. Ghayur and Gilani reported in 2005 that the crude extract of ginger induced a dose-dependent (0.3–3 mg/kg) fall in the arterial blood pressure of anesthetized rats. In Guinea pig paired

atria, the crude extract exhibited a cardiodepressant activity on the rate and force of spontaneous contractions. (Ghayur *et al.,* 2005)

It is proved that acetone extract of ginger and its constituents enhance the gastric emptying of charcoal meal in mice (Yamahara et al., 1990) and [6]-gingerol, [6]-shogaol, and galanolactone have anti- serotonin effects in isolated guinea pig ileum preparation (Huang et al., 1991). This may possibly suggest that the anti-emetic action mediated centrally via 5-HT3 receptors, as these constituents have small molecular weights and could easily cross the blood brain barrier. Ginger has been recorded as being useful in preventing post-operative nausea and vomiting in humans without a significant effect on gastric emptying (Phillips *et al.,* 1993).

Mahady et al., in 2003 were the first to provide evidence that the active constituents of ginger (gingerols) are effective in vitro against Helicobacter pylori, the primary etiological factor ssociated with dyspepsia, peptic ulcer disease and development of gastric and colon cancer.

The radioprotective effect of the hydroalcoholic extract of ginger rhizome was studied in mice given the extract at an intraperitoneal dose of 10 mg/kg, once daily for five consecutive days before exposure to 6–12 Gy of gamma radiation, and were monitored daily up to 30 days post-irradiation for the development of signs of radiation sickness and mortality (Jagetia *et al.,* 2003).

It has been shown that [6]-gingerol is endowed with strong anti-oxidant action both in vivo and in vitro, in addition to strong anti-inflammatory and anti-apoptotic actions (Kim *et al.,* 2007). Ginger extract (10 mg/kg) intraperitoneally had a dose dependent anti-microbial activity against Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli and Candida albicans.

Ginger-Drug Interactions: Few ginger–drug interactions have been reported in the literature. Ginger does not interact with the anti-coagulant drug warfarin in rats or man (Weidner and Sigwart, 2000). The synergistic effect of ginger and nifedipine on antiplatelet aggregation in normal human volunteers and hypertensive patients has been studied in Taiwan (Young et al., 2006).

C) *Boswellia serrata* Roxb.

It is a deciduous medium sized tree, with ash coloured bark, peeling off in thin flakes, shoots are young and leaves pubescent. Leaves are long, opposite, sessile variable in shape ovate or lanceolate, obtuse flowers in auxiliary racemes, shorter than leaves. Calyx is pubescent outside. Petals long and ovate. Drupe is tringonous. The tree is common at the foot of the Western Himalayas, in Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Bihar, Orissa, Andhra Pradesh and further south in the peninsular. In many places, the tree forms almost pure forests yielding an abundant supply of timber. Large forests of this tree occur in maharashtra and Khandwa-Nimar Division in Madhya Pradesh and Adilabad in Andhra Pradesh.

Fig 3.5 Bark and Gum Resin of *B.serrata*

C.1 Taxonomical Classification

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Gerniales

Family: Burseraceae

Genus: *Boswellia*

Species: *serrata* Roxb

C.2 Synonyms

English: Indian frankincense, Indian olibanum tree, **Hindi:** Shallaki, **Sanskrit:** Salai gugul, **Gujarati:** Guggula, **Kannad:** Guggulamara, **Marathi:**

Dhupali, **Tamil:** Kumancam, **Telugu:** Guggilamu, **Urdu:** Kundur, Lobana. (The Wealth Of India, 1988)

C.3 Marcoscopical Characteristics of the Gum Resin

C.3.1 Colour and Appearance: Pale yellow to brownish colour covered with a white little dust, occurs in more or less ovoid or pear shaped sometimes stalactitic form, occasionally agglutinated into small masses. Tears are mostly 5- 25 mm long.

C.3.2 Odour: Fragrant balsmic order.

C.3.3 Taste: Aromatic and slightly bitter. (Mukherjee, 2008)

C.4 Chemical constituents of Gum Resin of *B.serrarta*

Higher terpenoids constitute the major fraction (25-35 %) of the oleo-gum-resin. The first terpenoids isolated is boswellic acid. Besides boswellic acid several other triterpenoids have also been isolated from the gum resin, include α -amyrins, 11-keto-a-boswellic acid, 3' hydroxyl urs-9, 11 – dien-24-oic acid, 3'-acetoxy urs-9, 11-dien-24-oic- acid. Tetracyclic triterpenoic acids are 3'- hydroxyl-tirucall-8, 24-dien-21-oic acid 3'- hydroxyl-tirucall-8, 24-dien-21-oic acid, 3-keto-tirucall-8, 24-dien-21-oic acid 3'-hydroxy-tirucall-8, 24-dien-21-oic acid 3-keto-tirucall-8, 24-dien-21-oic acid, and 3'- acetoxy tirucall-8, 24-dien-21-oic acid. (Handa, 1995)

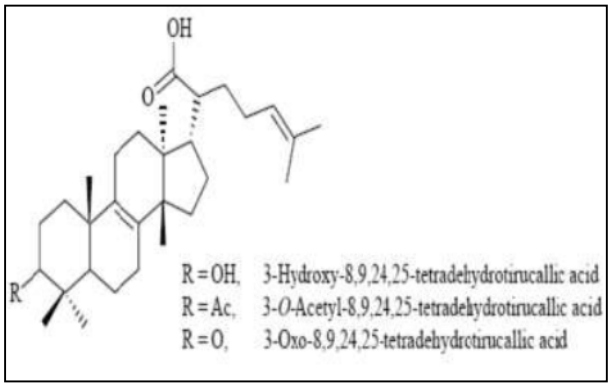


Fig 3.6a Structures of different Boswellic acids.

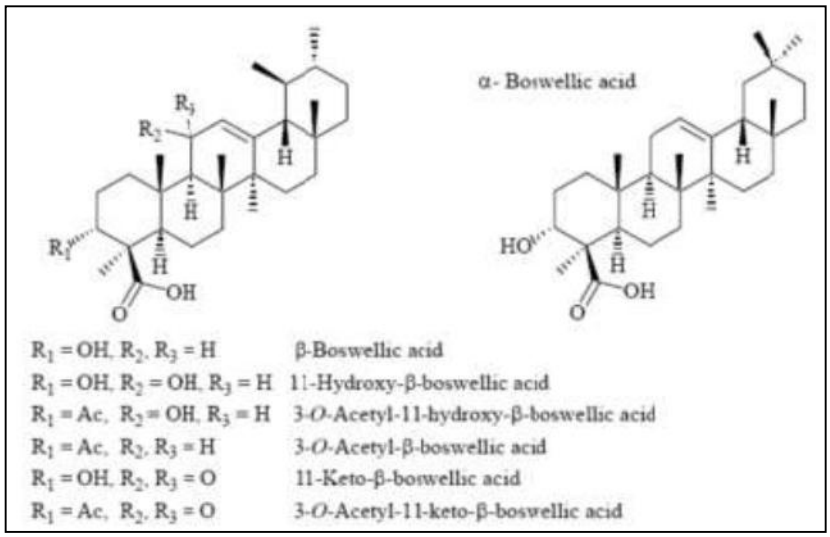


Fig 3.6b Structures of different Boswellic acids.

C.5 Traditional Uses

The oleo gum resin of *Boswellia serrata* is used in various Unani and Ayurvedic preparation. It is reported to be useful in the treatment of bronchitis, asthma, cough, bad throat and various intestinal problems. It is a diaphoretic and astringent prescribe in various syphilitic and pulmonary diseases. It acts as both internal and external stimulant, expectorant, diuretic and stomachic. The gum is also prescribed in cases of jaundice, diahhroea, dysentery, dyspepsia and hemorrhoids. It is also recommended in weak and unhealthy kind of ulceration. (Warrier *et al.,* 1994)

C.6 Pharmacological Properties

Extract of salai guggal caused inhibition of the Carrageenan induced rat hind paw oedema by 39.75% and 65-73%, administered orally in dose ranges of 50-200 mg per/kg and interaperitoneal (i.p.) in does range of 50-100 mg per kg-1 respectively compared to 47% inhibition seen with phenylbutazone (50mg/kg). In the anti-arthritic study on the mycobacterium adjuvant-induced poly-arthritic in rats, salai guggal showed 34% and 49% inhibition of paw swelling with 50 and 100 mg per/kg doses respectively as compared to controls. (Vernon, 1969)

Menon and Kar *et a*l, in 1970 revealed that the gum resin of *B. serrata* possess marked analgesic activity in experimental animals in addition to its sedative effect.

They have found that it produces reduction in the spontaneous motor activity and caused plosis in rats.

Singh and Atal in 1984 studied the anti-inflammatory activity of mixture of Boswellic acid (Composed of 5 acids with alpha Boswellic acid as the major component). This showed 25-46% inhibition of paw oedema in rats and mice. In chronic test of formaldehyde arthritis it exhibited 45-67% antiarthritic activity in a similar dose range. The fraction was effective in both adjuvant arthritis (35-59%) as well as established arthritis (54-84%). It also showed antipyretic effect, with no ulcerogenic effect and well tolerated in as high a dose as 2 gm/kg p.o, mice.

A study was carried out on leukocytes migration into the inflammatory exudates caused by Carrageenan. It was found to exert marked inhibitory effect on both the volume and leucocytes population of pleural exudates. In acute test model of Carrageenan induced pleurisy in rats extract of salai guggal in a dose of 100 mg per kg orally showed significant reduction of pleural exudates volume (47.93%): $P<0.001$) and leucocytes population (26.42% $P<0.001$). The effects on these parameters were more pronounced when animals were treated with Extract of salai guggal in a dose of 100 mg per kg for 10 days before the test performance. (Ammon *et al*., 1991)

The Boswellic acid when tested Papaya Latex Model, showed significant activity of mean 35% inhibition of inflammation. Since the model is reported to be sensitive to slowly acting, remission inducing drugs its effectiveness on boswellic acid throws some light on its mechanism of action which seems to be unlike aspirin and steroidal drugs. (Gupta O.P 1992).

Ethanol extract of the gum resin inhibits the formation of Leukotriene B_4 in rat peritoneal neurophils. Leukotriene such as LTB_4 is recognized as one of the important mediators of inflammatory reactions. Leukotrienes are synthesized by stimulated phagocytes cells, particularly the neutrophills. The production of chemostatic factors by these cells attracts more phagocytes to sites of inflammation. Most other non-steroidal anti-inflammatory drugs act through the inhibition of prostaglandins produced by stimulated phagocytes. Boswellic acid, therefore, is different from other

known non-steroidal anti-inflammatory drugs in its mode of action and relatively free from side effects (.naturalremedies.com).

Boswellic acids and derivatives concentration dependently decreased the formation of leukotriene B_4 from endogenous arachidonic acid in rat peritoneal neutrophils. Among the BAs, acetyl-11-keto-beta-boswellic acid induced the most pronounced inhibition of 5-lipoxygenase (5-LO). It did not impair the cyclooxygenase and 12-lipoxygenase in isolated human platelets and the peroxidation of arachidonic acid by Fe-ascorbate. (Safahyi *et al.,* 1992).

In a double blind placebo control clinical study with 300mg thrice daily dose for 6 weeks, it was established anti-asthmatic potential of alcohol extract of salai guggal where 70% of the patients with prolong history of asthma showed improvement in physical symptom and sign of dyspnoea, bronchi, number of attacks, increase in stimulation of mitogen activated protein kinase MAPK and mobilization of intracellular Ca^{2+} (Gupta *et al.,* 1998)

Acetyl 11-keto-β- boswellic acid is a novel highly specific inhibitor of 5-lipooxygenase. It inhibits 5-LO either by interacting with the enzyme directly or interacting with the 5-lipoxygenase activating proteins. (Bishnoi *et al.,* 2005.)

The different fractions of *B. serrata,* essential oil (10 ml/kg), gum (100 mg/kg, resin (100 mg/kg) oleo-resin (100 mg/kg) and oleo-gum-resin (100 mg/kg) shows analgesic activity, as determined by acetic acid induced writhing response, formalin induced pain, hot plate and tail flick method in which oleo-gum-resin fraction shows maximum inhibition (60.54) as compare to oil (20.70) and gum fraction (54.88). (Sharma *et al.,* 2010).

Extract of gum resin of B. serrata containing 60% acetyl 11-keto beta boswellic acid along with other constituents such as 11-keto beta-boswellic acid, acetyl beta-boswellic acid and beta-boswellic acid has been evaluated for antianaphylactic and mast cell stabilizing activity using passive paw anaphylaxis and compound 48/80 induced degranulation of mast cell methods. The extract inhibited the passive paw anaphylaxis reaction in rats in dose-dependant manner. However, the standard dexamethasone (0.27 mg/kg, p.o) revealed maximum inhibition of edema as

compared to the extract. A significant inhibition in the compound 48/80 induced degranulation of mast cells in dose-dependant manner was observed thus showing mast cell stabilizing activity. The standard disodium cromoglycate (50 mg/kg, ip) was found to demonstrate maximum per cent protection against degranulation as compared to the extract containing 60% AKBA. (Pungle *et al*., 2003).

Alcoholic extract of salai guggal for anti-carcinogenicity in mice with ehrlic ascites carcinoma and S-180 tumor, found inhibition of tumor growth by inhibiting cell proliferation and cell growth due to the interference with biosynthesis of DNA, RNA and proteins (Tsukada *et al.,* 1986). Topical application of Boswellic acid twice daily for 16 weeks to mice previously treated with dimethylbenz-anthracene caused 87- 99% inhibition in the number of tumor (Huang *et al.,* 1997).

Water soluble fraction of *B. serrata* extract decreased total cholesterol (38-48%) and increased high density lipoprotein in rats fed on atherogenic diet, thus proving its hypolipidemic potential (Zutsi *et al.,* 1986).

Boswellia serrata alcoholic extract was found effective in treating diarrhoea in patient with inflammatory bowel syndrome without causing constipation. The extract also inhibited gastrointestinal transit in croton and castor oil induced diarrhoea in mice. However, intestinal motility remained unaffected in control mice. (Borelli 2006).

C.7 Toxicity and Adverse Reactions: Four weeks toxicity study of Boswellic acids in rats in doses of 500 and 100 mg/kg orally and histology revealed no significant change in haematological and biochemical parameters and histology of vital organs. Acute oral and i.p. LD_{50} was greater than 2 g/kg. (naturalremedies.)

D) *Aegle marmelos* Corr.

A medium sized armed decidious tree upto 8.0 m high with straight sharp auxiliary thorns and yellowish brown shallowly furrowed corky bark. Flowers are large, bisexual, greenish-white, sweet scented, in short axillary panicles 4 to 5 cm long. Fruits are globose, grey, or yellowish, upto 20 cm in diameter, with woody rind; seeds numerous, oblong, compressed, embedded in sacs covered with thick, orange coloured sweet pulp. Root bark is 3 to 5 cm thick covered, with creamy yellowish surface. Stem bark is extremely gray and internally cream in colour. It is 4-8 mm thick, film in texture and occurs as flat or channeled pieces. Locally known as 'bael', is indigenous to India, grows wild throughout the deciduous forests of central and southern parts. It is considered highly religious since it is used extensively to worship gods specially for worshiping Lord Siva.

Fig 3.7 *Aegle marmelos*, whole plant and leaf

D.1 Taxonomical Classification

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Gerniales

Family: Rutaceae

Genus: *Aegle*

Species: *marmelos* Corr.

D.2 Synonyms

English: Elephant apple, Bael **Hindi:** Baelpatra, **Sanskrit:** Belli, bili, **Gujarati:** Bhishan, **Kannad:** Baelada mara, **Marathi:** Bel, Vel, **Tamil:** Kuuviram, Vilvama, **Telugu:**Maaredu, **Urdu:** Bel kham, Belgiri. (The Wealth of India, 1985)

D.3 Macroscopical Characteristics of Leaf

D.3.1 Colour and Appearance: Leaves are alternate, attenuate trifoliate, Petiole 2.5 to 6.3 cm long. Leaflets are ovate or ovate- lanceolate, margins crenate, apex acute, glabrous and densely minutely glandular-punctuate on both surfaces. Lateral leaflets to 7 cm long and 4.2 cm wide,

D.3.2 Odour: Characteristic.

D.3.3 Taste: Bitter (Chanda *et al.,* 2008).

D.4 Microscopical Characteristics of Leaf

Lamina in vertical section shows, single layered upper epidermis composed of polygonal barrel-shaped cells with straight thick anticlinal walls covered with thick cuticle, cells of lower epidermis smaller in size, stomata anomocytic, present on both the surfaces but abundant on lower surface; mesophyll differentiated into 2 or 3 layers of small palisade cells which are continuous on midrib and very compactly arranged spongy parenchyma containing chloroplast large, circular secretory cannals surrounded by single layer of epithelial cells present in mesophyll.

Midrib slightly pronounced towards lower surface; shows single layered epidermis covered with thick cuticle, cells of upper epidermis are bigger in comparison to lower surface; lower epidermis of midrib by 2 or 3 layers of collenchyma, remaining ground tissue parenchymatous; meristele arc shaped, vascular bundle consists of radially arranged xylem and encircled by phloem, pericycle represented by patches of sclerenchymatous fibres. Rare trichomes, found mainly on lower surface of mid-rib region, unicellular and 399 - 696 µm long. Stomata 62 - 68 µm in size, stomatal index of upper epidermis 4.5 - 9, lower epidermis 9.5 - 12.5; stomatal number of upper epidermis 200 - 400 per sq. mm, lower epidermis 300 - 600 per sq. mm; palisade ratio 1.25 - 2.5; vein-islet number 10 - 20 per sq. mm. (Tiwari *et al.,* 2010).

D.6 Traditional Uses:

The leaves of this plant are used in indigenous system of medicine as astringent, laxative, expectorant, in the treatment of various gastro-intestinal affections (dysentery and piles), ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation, and asthmatic complications. The leaves are also reported in the treatment of abscess, backache, diabetes, eye disorders, fever, heat in abdomen, jaundice, vomiting, wounds and cuts, while contraceptive property of the drug is also been claimed. Fresh aqueous and alcoholic leaf extracts are reported to have cardiotonic effects in mammals; said to possess hypoglycemic and anti-hyperglycemic activity, anti spermatogenic activity, antioxidant activity and anticancer effect. (Siddique *et al.,* 2010)

D.7 Chemical Constituents

Several compounds have been isolated from the leaves such as skimmianine, aegeline, lupeol, cineole, citral, citronellal, cuminadlehyde, egenol and marmesinin d-limonene. Sugars and proteins are also present. (Maity *et al.,* 2009)

D.8 Pharmacological Properties

Veerappan et al., in 2004 proved in a comparative study of different extract of dried leaves that methanolic extract showed maximum inhibition of 70.97% at the dose of 50 mg/kg in Carageenan induced paw oedema, 47.57 % in sub-acute studies using model cotton-pellet granuloma in rats, 68.20% inhibition in Acetic acid induced-writhing in mice and 96% inhibition in yeast induced hyperpyrexia in mice.

The aqueous extract of leaves have also shown significant hypoglycemic and antioxidant activity in alloxan induced male albino diabetic rats. (Upadhyay *et al.,* 2004).

Pre-treatment with Aegle marmelos leaf extract (AMLEt) at doses of 100 mg/kg and 200 mg/kg bodyweight for 35 days showed a significant effect on the activities of marker enzymes, lipid peroxides, lipids, lipoproteins and antioxidant enzymes in isoproternol-treated rats. The effect of AMLEt 200 mg/kg was found to be equal to the effect of alpha-tocopherol 60 mg/kg. It concludes that *Aegle marmelos* leaves

possess antihyperlipidaemic effect in rats with ISO-induced myocardial infarction. (Rajadurai and Prince, 2005)

The effect of hydroalcoholic (80% ethanol, 20% water) extract of leaves of *Aegle marmelos* was examined on carcinogen-metabolizing phase-I and phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation, using two doses of dried extract (50 and 100 mg kg^{-1} daily for 14 days), in the liver of mice. The modulatory effect of the extract was also examined on extrahepatic organs (lung, kidney and fore-stomach) for effects on the activity of glutathione S-transferase, DT-diaphorase, superoxide dismutase and catalase. Extract treatment significantly increased the basal levels of acid-soluble sulphydryl (–SH) content, cytochrome P_{450}, NADPH-cytochrome P_{450} reductase, cytochrome b_5, NADH-cytochrome b_5 reductase, glutathione S-transferase, DT-diaphorase, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the liver. (Singh *et al.,* 2000)

It was observed that methnolic extract of leaves of *A. marmelos* at 100 ng/ml concentration showed complete loss of motility of microfilariae using *Brugia filariae* after 48 hours of incubation. (Sahare *et al.,* 2008).

The ethanolic extract of leaves shows significant lipid lowering activity in hyperlipidemic models of Wistar albino rats at doses of 125 mg/kg and 250mg/kg. (Vijaya *et al.,* 2009)

Rana *et al*., in 1997 evaluated the anti fungal activity of essential oil isolated from leaves of the *Aegle marmelos* using spore germination assay. The oil established variable efficacy against different fungal isolation and 100% spore germination off all the fungi tested and observed at 500 ppm. However the most resistant fungus, F*usarium udum* was inhibited by 80% at 400 ppm.

Bael acted as a bifunctional inducer since it induced both phase-I and phase-II enzyme systems. Both doses significantly decreased the activity of lactate dehydrogenase and formation of malondialdehyde in liver, suggesting a role in cytoprotection as well as protection against pro-oxidant-induced membrane damage. Butylated hydroxyanisole (positive control) induced almost all the antioxidative

parameters measured in this study. The extract was effective in inducing glutathione S-transferase, DT-diaphorase, superoxide dismutase and catalase in lung, glutathione S-transferase, DT-diaphorase and superoxide dismutase in fore-stomach, and DT-diaphorase and superoxide dismutase in lung. These significant changes in the levels of drug-metabolizing enzymes and antioxidative profiles are strongly indicative of the chemopreventive potential of this plant, especially against chemical carcinogenesis (Singh *et al.,* 2000).

Free radical scavenging activity of methanolic extract of different parts (leaf bark and stem) was evaluated by using DPPH method. The highest free radical scavenging effect was observed in leaves with IC50 of 2.096µg per ml. The effectiveness of radical scavenging activity of leaves extract was about 10 times greater than reference antioxidant butylated hydroxy toluene (BHT). The greater amount of phenolic compounds leads to more powerful radical scavenging effect as shown by methanolic extract of *A. marmelos* leaves. (Siddique *et al.,* 2010)

The Bael fruit also has been reported for anti-diabetic activity, cardioprotective effects, anti-spermatogenic activity, antimicrobial/antifungal activity, antiulcer activity, anti cancer activity, antipyretic and analgesic activity, radioprotective effect, respitory infections,constipation and diarrhea.(Sharma *et al.,* 2007).

E) Medicinal Plants Having Anti-Arthritic Potential.

Table 3.1 Medicinal Plants Possessing Anti-Arthritic Activity

Name of the Plant	Part used	Model	Reference
Terminalia paniculata	Stem bark	CIPO,API,CFAIA	Talwara *et al.,* 2011
Alpinia galanga	rhizome	AIA	Chandur *et al.,* 2010.
Sophora flavescens	Roots	AIA	Jin *et al.,* 2010
Cassia siamea	Stem bark	Hot plate test, paw pressure, CIPO	Ntandoua *et al.,* 2010
Alpinia officinarum	rhizome	CFAIA	JiSuk *et al.,* 2009
Albizia lebbeck	Stem bark	CIPO,CPG,CAIA	Babua *et al.,* 2009
Centella asiatica	Leaf	C(II)IA	Mei 2008.
Euphorbia tirucalli	Whole plant	AIA	Bani *et al.,* 2007
Cleome gynandra	Leaf	AIA	Narendhirakannan *et al.,* 2007
Ginkgo biloba	Isolated terpene	CACAI	Yongmoon 2005.
Baccharis genistelloides	Aerial parts	CIA	Coelho *et al.,* 2004
Trewia polycarpa	Roots	AIA	Chamundeeswari *et al.,* 2003
Argyreia speciosa	Leaf	CIPO,AIA	Gokhle *et al.,* 2002
Alstonia boonei	Stem bark	CIPO, CPG	Olajide *et al.,* 2000
Cedrus deodara	Wood oil	CIPE, FAIA	Shinde *et al.,* 1999
Hippocratea excelsa	Leaf	CIPO, CPG,AIA	Perez *et al.,* 1995

AIA- Aduvant induced arthritis; CIA- Collagen induced arthritis; CPG- Cotton pellet Granuloma; CIPO- Carrgeenan induced paw odema; CACAI- Candida albicans caused arthritis inflammation; API- air pouch inflammation

Some other important plants possessing anti-arthritic activity are Andrograhis paniculata, Allium sativum, Apium graveolens, Aspargus racemosus, Boerhaavia

Diffusa, Celastrus paniculatus, Cissus quandrangularis, Commiphora weightii Curcuma longa, Ficus Benghalensis, Gmelina Arborea, Haldinia Cordifolia, Melia Azaderach, Moringa Pterygosperma, Papaver somniferum, piper longum, Plumbago Zeylanica, Ricinus communis, Tinospora Cordifolia, Tylophora Asthematica Valerinina Wallichii, Vanda roxburguii, Withania somnifera.

F) Carbopol:

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are produced from primary polymer particles of about 0.2 to 6.0 micron average diameter. The flocculated agglomerates cannot be broken into the ultimate particles when produced. Each particle can be viewed as a network structure of polymer chains interconnected via cross-linking. (Florence et al., 1994)

Carbomers readily absorb water, get hydrated and swell. In addition to its hydrophilic nature, its cross-linked structure and its essentially insolubility in water makes Carbopol a potential candidate for use in controlled release drug delivery system. (Carnali *et al.,* 1992)

They are offered as fluffy, white, dry powders (100% effective). The carboxyl groups provided by the acrylic acid backbone of the polymer are responsible for many of the product benefits. Carbopol polymers have an average equivalent weight of 76 per carboxyl group.

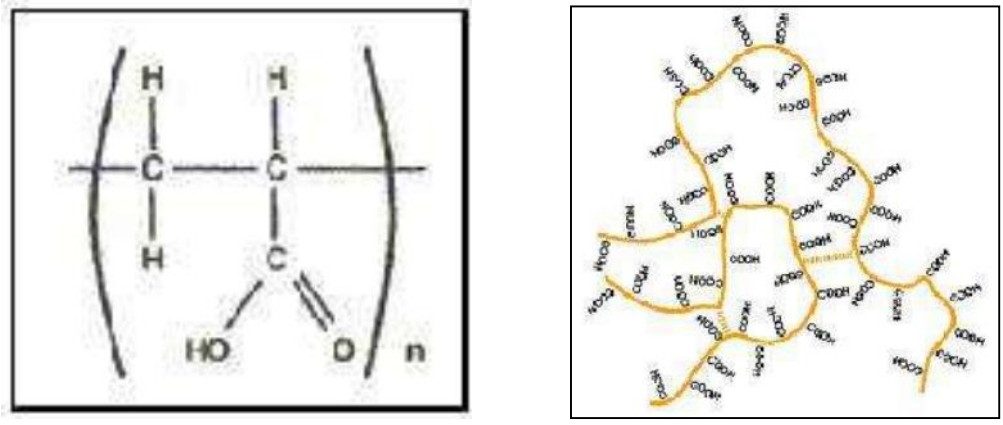


Fig 3.8 a. General Structure of Carbopol Polymers. b. Schematic drawing of a molecular segment of a cross-linked polyacrylic acid polymer

Carbopol 934 P is cross-linked with allyl sucrose and is polymerized in solvent benzene. The three dimensional nature of these polymers confers some unique characteristics, such as biological inertness, not found in similar linear polymers. The Carbopol resins are hydrophilic substances that are not soluble in water. Rather, these polymers swell when dispersed in water forming a colloidal, mucilage-like dispersion.

Carbopol polymers are bearing very good water sorption property. They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0. Because the pKa of these polymers is 6.0 to 0.5, the carboxylate moiety on the polymer backbone ionizes, resulting in repulsion between the native charges, which adds to the swelling of the polymer. The glass transition temperature of Carbopol polymers is 105°C (221°F) in powder form. However, glass transition temperature decreases significantly as the polymer comes into contact of water. The polymer chains start gyrating and radius of gyration becomes increasingly larger. Macroscopically, this phenomenon manifests itself as swelling.

Applications of Carbopol Polymers:

The readily water-swellable Carbopol polymers are used in a diverse range of pharmaceutical applications to provide:

- Controlled release in tablets.
- Bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal and rectal applications.
- Thickening at very low concentrations to produce a wide range of viscosities and flow properties in topical, lotions, creams and gels, oral suspensions and transdermal gel reservoirs.
- Permanent suspensions of insoluble ingredients in oral suspensions and topicals.
- Emulsifying topical oil-in-water systems permanently, even at elevated temperatures, with essentially no need for irritating surfactants

Topical Applications:

Carbomers are very well suited to aqueous formulations of the topical dosage forms. Many commercial topical products available today have been formulated with these polymers, as they provide the following numerous benefits to topical formulations:

- Safe & Effective — Carbopol polymers have a long history of safe and effective use in topical gels, creams, lotions, and ointments. They are also supported by extensive toxicology studies.
- Non-Sensitizing — Carbopol polymers have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage.
- No Effect on the Biological Activity of the Drug — Carbopol polymers provide an excellent vehicle for drug delivery. Due to their extremely high molecular weight, they cannot penetrate the skin or affect the activity of the drug.
- Excellent Thickening, Suspending, & Emulsification Properties for Topical Formulations.

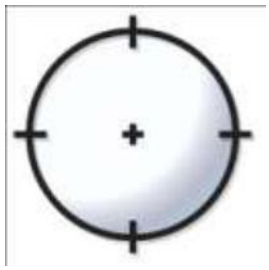
Toxicological Studies:

The Carbopol, like other high molecular weight polymers, demonstrate a low toxic and irritation potential based on their physical and chemical properties. Acute oral studies with rats, guinea pigs, mice and dogs showed that Carbomers- 910, -934, -940 and –941 have low toxicities when ingested.

The inhalation LC_{50} of Carbomers 910 in albino rats was 1.71mg/l. the dermal LD_{50} of rats exposed to Carbopol 910 was greater than 3.0 g/kg. No mortalities occurred in rabbits injected intravenously with 1%, 2% or 3% Carbopol 934 in aqueous solution at a dose of 5 ml/kg. Rabbits showed minimal skin irritation when tested with 100% Carbopol 910 or –934, and zero to moderate eye irritation when tested with Carbomers 910, -934, -940, -941 and/or their various salts at concentrations of 0.20-100%. When Carbopol 934 P was fed orally to dogs and rats, there was no significant effect on body weight, food consumption, mortality, behavior, and blood chemistry.

G) Marketed Preparations

The anti-rheuatic drugs by Charak pharmaceuticals are Traquinyl Forte tablets, Rymanyl tablets, Rymanyl liniment, Manoll tonic, Manoll tablets. Anti-rheumatic preparations by Himalaya are Gertiforte syrup/ tablets, Mentat (Syrup/ tablets), Rumalaya cream/ tablets, Septilin syrup/tablets., Serpina tablets.



Chapter 4

Aim and Objectives

4. AIM AND OBJECTIVES

Aim

Formulation and evaluation of a herbal formulation for arthritis.

Objectives

1. To prepare and evaluate the formulation and its optimization.
2. To carry out anti-inflammatory activity of the gel.
3. To carry out the anti-arthritic activity of the gel.
4. To carry out the antioxidant activity of the drug extract in the combined form.
5. To estimate 6-gingerol in gel.

Chapter 5

Materials and Methods

5. MATERIALS AND METHODS

A) Plant Materials and Standard Drugs

The drugs were procured from Lalubhai Vrijlal Gandhi and Nirma Herbal Wealth, Nirma University, Ahmedabad (Gujarat). The drugs were authenticated by Dr. Bhaskar L. Punjani, Head, P.G. Center in Botany, Smt. S.M. Panchal Science College, Talod, (Dist Sabarkantha), Gujarat. The voucher specimen **IPS/PCOG/MPH10-11/010/509** was submitted to Institute of Pharmacy, Nirma University. The drugs were powdered and stored in airtight plastic container at room temperature until needed.

B) Chemicals

All the solvents were procured from CDH chemicals, Fine chemicals and standard of 6-Gingerol was procured from Natural Remedies Pvt. Ltd, Bangalore.

C) Preliminary Evaluation

C.1 Morphology and Microscopy

The morphology of the dried ginger powder, gum resin of *B. serrata* and fresh leaf of *A. marmelos* was done. The microscopy of fresh leaf of *A. marmelos* was done. The sections were cleared with sodium hypochlorite solution and stained with phloroglucinol and the pictures were taken with the help of charged chemical device camera attached with microscope (Lawrance and Mayo Ltd).

C.2 Physical Profile (Quality Control Method for Medicinal Plant Materials, 2002.)

C.2.1. Determination of Foreign Matter

100–500 g of the all 3 drug samples to be examined was weighed and spread on a thin layer. The foreign matters have been examined by the use of a lens.

C.2.2. Determination of Total Ash

About 2 g of the ground air dried material, accurately weighed drug, in a previously ignited and tared crucible. The material was spread in an even layer and was ignited by gradually increasing the heat to 500-600 ^{o} C until it was white, indicating the absence of carbon. It was cooled in desiccators and weighed. The percentage of ash with reference to the air-dried drug was calculated.

C.2.3 Determination of Acid Insoluble Ash

The ash obtained in C.2.2 was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water until the filtrate became neutral and was ignited to constant weight in the crucible. The percentage of acid-insoluble ash with reference to the air dried drug was calculated.

C.2.4. Determination of Alcohol Soluble Extractive value

4 g of the air dried drug, coarsely powdered, accurately weighed was taken in a glass-stoppered conical flask. 100 ml of alcohol was added and weighed again to obtain the total weight including the flask and was allowed to stand for 1 hour after shaking. A reflux condenser was attached to the flask and was boiled gently for 1 hour. It was cooled and weighed and the weight was readjusted to the original weight with the alcohol and was filtered. 25 ml of the filtrate was transferred to a tared flat bottom dish and was evaporated to dryness on a water bath. It was dried for 6hours at 105˚C and was cooled in a desiccator for 30 minutes. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

C.2.5. Determination of Water Soluble Extractive value

4 g of the air dried drug, coarsely powdered, accurately weighed was taken in a glass-stoppered conical flask. 100 ml of distilled water of the specified strength was added and was weighed again to obtain the total weight including the flask and was allowed to stand for 1 hour after shaking. A reflux condenser was attached to the flask and was boiled gently for 1 hour. It was cooled and weighed and the weight was readjusted to the original weight with the water and was filtered. 25 ml of the filtrate was transferred to a tared flat bottom dish and was evaporated to dryness on a water bath. It was dried for 6hours at 105˚C and was cooled in a desiccators for 30 minutes. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

C.2.6 Determination of Loss on Drying

4 g of the air dried drug, coarsely powdered, accurately weighed was taken in a flat bottom dish and was again weighed. It was kept in hot air oven at 100℃ and was

weighed after every 30 minutes until the weight was found to be constant. The percentage of LOD with reference to the air-dried drug was calculated.

C.3 Phytochemical screening (Khandelwal, 2008)

C.3.1. Tests for Alkaloids

The hydroalcoholic (1:1) extract of Z. officinalis, methanolic extract of B. serrata and A. marmelos were prepared and were subjected to different tests.

(i) Mayer's Reagent: Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added separately to each extract and observed for the formation of white or cream colored precipitates.

(ii) Dragendroff's Reagent: Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added separately to each extract and observed for the formation of orange yellow precipitates.

(iii) Hager's Reagent: Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added separately to each extract and observed for the formation of yellow precipitates.

(iv) Wagner's Reagent: Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added separately to each extract and observed for the formation of reddish brown precipitates.

C.3.2 Tests for Carbohydrates

(i) Molisch's Test: A small amount of each extract was dissolved in ethanol and two drops of a 20% w/v solution of α-napthol in ethanol were added to it. Now, about 1 ml of concentrated H_2SO_4 was slowly added along the sides of the test tube. Appearance of red-violet ring at the junction of the two layers indicated the presence of carbohydrates.

(ii) Fehling's Test: A small amount of the each extract was dissolved in about 2 ml of distilled water and filtered. An equal amount of Fehling's solution was added to the filtrate and the contents were boiled. Appearance of brick red precipitates confirmed the presence of reducing sugars.

(iii) Benedict Test: A small amount of the each extract was dissolved in about 2 ml of distilled water and filtered. About 1 ml of Benedict solution was added to the filtrate. The contents were boiled and observed for the appearance of brick-red precipitates which confirmed the presence of reducing sugars.

C.3.3 Tests for Glycosides

(i) Borntrager's Test: A small amount of each extract was hydrolysed with dilute HCl for a few min on water bath. To the hydrolysate, about 1.0 ml of benzene and 0.5 ml of dilute ammonia solution was added. Appearance of reddish-brown color at the junction of the two layers confirmed the presence of glycosides.

C.3.4 Tests for Sterols

(i) Liebermann-Burchard's Test: A small amount of each extract was dissolved separately in chloroform and few drops of acetic anhydride were added. Now, concentrated sulphuric acid was added drop-wisely along the sides of the test tube and observed for the appearance of blue to blood red color as the indication of sterols.

(ii) Salkowski Test: A small amount of each extract was dissolved in chloroform. Concentrated sulphuric acid was added dropwise along the sides of test tube and observed for presence of red or yellow colour at lower layer.

C.3.5. Tests for Saponins

(i) Foam Test: About 1 ml of each extract (in the respective solvents) was separately diluted to 20 ml with distilled water and further shaken in a graduated cylinder for 15 minutes. Formation of about 1 cm thick layer of foams confirmed the presence of saponins.

C.3.6 Tests for Phenolic Compounds and Tannins

(i) Ferric Chloride Test: Small amount of each extracts were separately shaken with water and warmed. Now about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue color.

(ii) Lead Acetate Test: A few milligrams of each extract were separately stirred with about 2 ml distilled water and filtered. To the filtrate, few drops of 10% w/v lead acetate solution was added and observed for the formation of white precipitates.

C.3.7 Tests for Amino Acids and Proteins

(i) Millon's Test: A small amount of each extract was separately dissolved in about 5 ml of distilled water and filtered. To 2 ml of the each extract, 5-6 drops of Million's reagent (solution of mercury nitrate and nitrous acid) were added and observed for formation of red precipitates as an indication of the presence of proteins.

C.3.8 Tests for Flavonoids

(i) Shinoda Test: A few milligrams of each extract were separately shaken with ethanol in different test tubes. Now, small pieces of metallic magnesium or zinc were added followed by addition of 2 drops of concentrated HCl and observed for the formation of pink color.

(ii) Aqueous NaOH Test: To test solution add 10 % NaOH, yellow color is obtained.

(iii) Mineral Acid Test: To test solution add conc. H_2SO_4, yellow-orange color is obtained.

(iv) Lead Acetate Solution Test: To the test solution add 10 % of lead acetate solution, yellowish precipitates are obtained.

D) Preparation and TLC of the Extracts

The powdered drugs were sieved through 40 # mesh sieve. The measured amount of the powder of *Zingiber officinalis* and *Boswellia serrata* were then subjected to continuous and sequential solvent extraction using methanol : water (1:1) and methanol respectively in soxhlet apparatus. The extract of *Aegle marmelos* was also prepared using methanol as a solvent using soxhlet assembly. All the filtrates were concentrated and used in the formulation.

D.1 TLC of Extracts

Stationary phase: Silica gel 60 F254

Solvent system: 1*) Z. officinalis* - Hexane-Ether (4:6)

2) *B. serrata* - Hexane- Ethylacetate (7:3)

3) *A. marmelos* – Toluene : Ethylacetate : Formic acid (7:3:0.1)

E) *In-vitro* Antioxidant Activity

All extracts were subjected to antioxidant activity individually as well as in combined form. They are combined in the ratio of 1:1:1.

DPPH Free Radical Scavenging Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by spectrophotometric method. 2 ml of methanolic solution of the extracts of various concentrations (0-100 µg/ml) were mixed with 1 ml of ethanolic solution of DPPH (0.1 mM). A mixture of 2 ml of ethanol and 1 ml of ethanolic solution of DPPH served as control. After mixing, all the solutions were incubated in dark for 20 min and then absorbance was measured at 517 nm and IC_{50} was measured. (Stoilova, 2007). The % inhibition of free radicals was measured by the following equation:

$$\% \text{ inhibition} = [A_0 - A_1/A_{0]} \times 100$$

A0 = Absorbance of control
A1 = Absorbance of test solution

F) Development of Formulation

F.1 Selection of Polymer

For the selection of polymer six different polymers in different concentrations were used. They were added to water in small quantities with continuous stirring with a help of stirrer. The stirring was continued until a homogenous mixture was formed.

Table 5.1 Polymers and their concentrations

Name of Polymer	Concentration
Hydroxy propyl methyl cellulose 50 cps	5%
Polyethylene oxide 303	1%
Sodium caboxymethylcellulose	5%
Sodium alginate	5%
Carbopol 940	1.5%
Carbopol 934	1%
Solvent – water	

F.2 Optimization of Concentration of Carbopol 934.

The optimization of the gel was done by using different concentration of the polymer carbopol 934.

Table 5.2 List of ingredients used in formulation of gel using Carbopol 934

Phytoconstituents	0.5 gm of extract of *Zingiber officinalis* 0.5 gm of extract of *Boswellia serrata* 0.5 gm of extract of *Aegle marmelos*
Polymer	Carbopol 934 (.4%) Carbopol 934 (.5%) Carbopol 934 (1%) Carbopol 934 (2%)
Solvent	Water (30 ml)
Solvent to dissolve extracts	*Zingiber officinalis*- isopropyl alcohol+ water(1+1 ml) *Boswellia serrata* – isopropyl alcohol (2 ml) *Aegle marmelos* – isopropyl Alcohol (2 ml)
pH enhancer	Triethanolamine (q.s)
Preservative	Methyl paraben- 0.03 gm

F.3 Procedure for the Preparation of Gel.

Step 1: Take 20 ml of distilled water in a 100 ml beaker and the weighed preservative was dissolved.

Step 2: Required quantity of the polymer was weighed.

Step 3: The polymer was added to the water in small quantities with continuously stirring on a stirrer. (The stirring was continued until the polymer dispersed homogenously).

Step 4: The colloidal mixture formed was allowed to stand for few minutes to remove air bubbles (sonicate if any bubbles are remained).

Step 5: 0.5 gm of prepared extracts of *Zingiber officinalis, Boswellia serrata* and *Aegle marmelos* was weighed separately. Ginger extract was dissolved in 2 ml of isopropyl alcohol and water mixture (1:1), other two were dissolved in 2ml isopropyl alcohol separately.

Step 6: The three mixtures of the drugs were mixed colloidal dispersion of the polymer and stirred slowly with the help of a glass rod and measure the pH

Step 7: Triethnolamine was added drop wise. After each addition the mixture was stirred with a glass road. (Caution: higher amount of TEA liquefies the gel).

Step 8: The remaining amount on water (4 ml) was added and gel was transferred into the container.

F.4 Evaluation Parameters

The evaluation parameters carried out for gel are:

- Measurement of viscosity by Brookfield viscometer using T96 at 100 rpm.
- Smoothness and Spreadbility of the gel
- Gel extrusion by texture analyzer using Texture Pro v2.1 software in which adhesive force, Adhesiveness, peak load, mean load final load were calculated by using probe 40.

  The conditions were:

  Test type: Compression

  Trigger point: 5 g

  Target value: 30mm

  No. of cycles: 1

  Test speed: 30mm/min

Standard: Piroxicam Gel BP 0.5% (Cipla pvt Ltd)

G) *In-vivo* Study

G.1 Animals

Healthy adult male albino rats of Sprague Dawley strain weighing between 200-250 gm were selected for the study. Animals were maintained at 25 ± 2^{o}C and kept in well ventilated animal house under natural photoperiodic condition in polypropylene cages with free access to food and water *ad libitum*. During the period of experiment the animals were fed with the standard rat diet. Animals were acclimatized for 10 days before starting the study.

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute Of Pharmacy, Nirma University, Ahmedabad and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Protocol number is **IPS/PCOG/MPH10-11/010.**

G.2 Carrageenan Induced Paw Edema

Table 5.3 Grouping of animals for anti-inflammatory activity

Serial No.	Groups	No. of animals
1	Control – base treated	5
2	Formulation- treated	5
3	*Z. officinalis* gel- treated	5
4	*B. Serrata* gel-treated	5
5	*A. marmelos* gel-treated	5
6	Standard	5

Animals were fasted for 24 h before the experiment with free access to water. Approximately 50 ml of a 1% suspension of carrageenan (Sigma Co., USA) in saline was prepared 1 h before experiment and was injected into the plantar side of right hind paw of rat. 0.2 g of gel containing 5% of extracts and individual gel of each extract were applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger. Rats of the control groups received only the gel base. Piroxicam gel BP 0.5% (Cipla pvt Ltd) applied in the same way was used as a reference. Drugs or placebo were applied 1 h before the carrageenan injection (0.05ml). Paw volume was measured immediately after carrageenan injection and at 1-, 2-, 3- and 4-h

intervals after the administration of the noxious agent by using plethysmometer (PTH 707 Digital Volume Meter, Medicad). (Niemegeer *et al.,* 1964). The % inhibition is of paw volume is calculated by the following equation:

% inhibition = [control – treated/ control] x 100

H) Adjuvant Induced Developing Arthritis in Rats

Animals were immunized with an injection of 50 µl of 5 mg/ml (w/v) suspension of heat killed *Mycobacterium tuberculosis* (Complete Freund,s Adjuvant) in liquid paraffin into the left hind foot in the subplantar region. 0.2 gm of gel application was started a day before the immunization to three treatment groups and continued till day 13. Group III received piroxicam (0.2 mg/paw) in similar manner whereas Group I received gel base only. Paw volume was measured on day 0 and every alternate day till day 13 with volume Digital Volume Meter PTH 707, Medicad. (Newbould, 1963). The % inhibition is of paw volume is calculated by the following equation:

% inhibition = [control – treated/ control] x 100

Table 5.4 Grouping of animals for anti-arthritic activity

Serial No.	Groups	No. of animals
I	Control	6
II	Treated	6
III	Standard	6

I) Estimation of 6-Gingerol in Formulation by HPTLC

I.1 System:

- Camag Linomat 5
- Semiautomatic application, band application by spray on technique (2 - 500µl)
- Camag twin trough glass chamber (10 x 10 and 20 x 10)
- Camag TLC scanner 3
- Scanning speed upto 100mm/s, Spectral range 190 – 800nm
- Camag Reprostar 3 with digital camera
- Camag UV cabinet with dual wavelength UV lamp

- Dual wavelength 254 / 366nm
- Stationary Phase: Silica gel G60 F_{254} coated on aluminum sheet.
- Hamilton 100µl HPTLC syringe.

The analysis was performed with HPTLC (Camag, Switzerland). The standard solution of 6-Gingerol and samples were applied with the Linomat III applicator on the HPTLC silica gel 60 F_{254} plate (E. Merck, Germany). The plates were developed with a twin-trough developing chamber. After development, the plates were scanned with a Camag TLC scanner 3, and the data were processed with WINCATS software.

I.2 Preparation of Test Sample and Standard solution: 1 gm of gel was weighed accurately and was dissolved in 50 ml of methanol. It was filtered and the residue was again dissolved in 25 ml of methanol and filtered again. The two filtrates were combined and evaporated to dryness and was redissolved in 1 ml of methanol. (Gupta *et al.,* 2010)

The standard solution was prepared using solvent methanol of concentration 50ng/ml. The standard solution was applied in the range from 200 ng to 1400 ng. and sample solution applied on the plate were 5 µl and 10 µl.

I.3 Chromatographic Conditions:

Stationary phase: Silica gel 60 F254

Solvent system: Hexane : Ether (4:6)

Band width: 4mm

Solvent front: 8.5 cm

Scanning: 254 and 366 nm

Spraying reagent: Vanillin-Sulphuric Acid reagent

Drying Device: hair dryer

Slit dimensions: 5x.30 mm.

Chapter 6

Results

6. RESULTS

A) Preliminary Pharmcognostical, Physical and Phytochemical Profile

A.1 Morphology

A.1.1 Morphology of dried powder of *Z. officinalis*

Light brown in colour, fine and smooth powder with characteristic smell and pungent taste.

Fig 6.1 Ginger powder

A.2.1 Morphology of gum *B. serrata*

Pale yellow to brownish colour covered with a white little dust, occurs in more or less ovoid or pear shaped sometimes stalactitic form, occasionally agglutinated into small masses. Tears are mostly 5- 25 mm long.

Fig 6.2 Gum resin of *B. serrata*

A.1.3 Morphology of leaf of *A. marmelos*

Pale green, compound, smooth, alternate, odd pinnate of 3 leaflets. Lateral leaflets opposite and nearly sessile, ovate-lanceolate, 4.5 - 8.5 cm long and 2 – 5 cm broad. The terminal leaflet with long rachis, 7 - 13 cm long and 3.5 - 7.5 cm broad, ovate-lanceolate, entire or crenate, acute, rachis 1.5 - 3 cm long, petiole not winged about 3 - 8 cm long.

Fig 6.3 Leaf of *A. marmelos*

A.2 Microscopy of Leaf of *Aegle Marmelos*

The pictures were taken through CCD camera attached with microscope.
1µm = 0.5 cm for 10 x and 1 µm = 1.8 cm for 45 x

A.2.1 T.S of Leaf

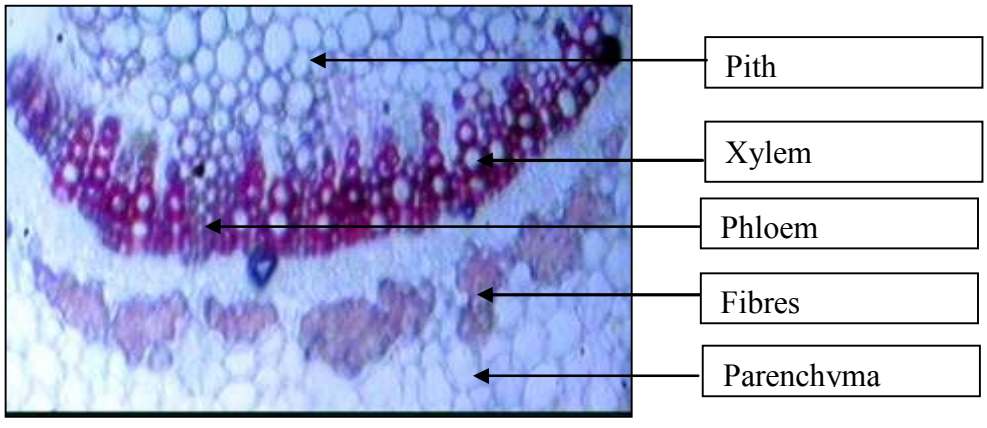


Fig 6.4 T.S of Leaf Stained with Phloroglucinol at 200x

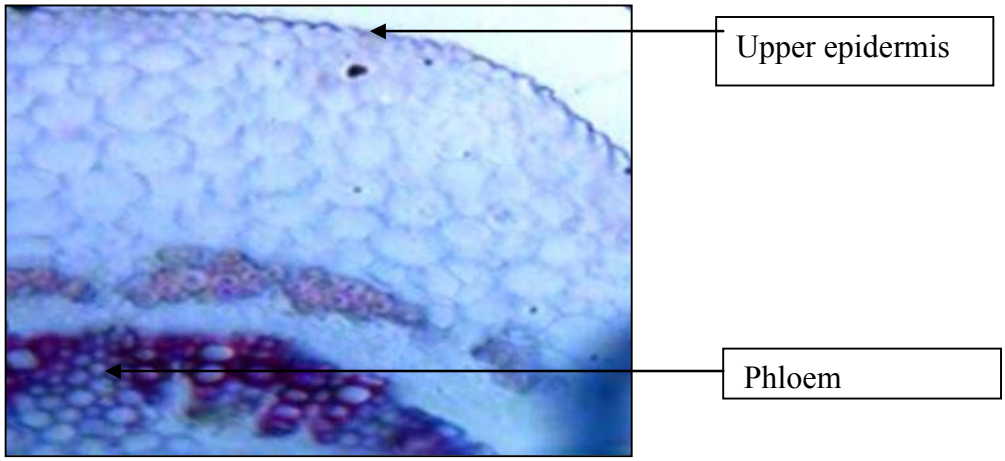


Fig 6.5 T.S of leaf stained at 500 x

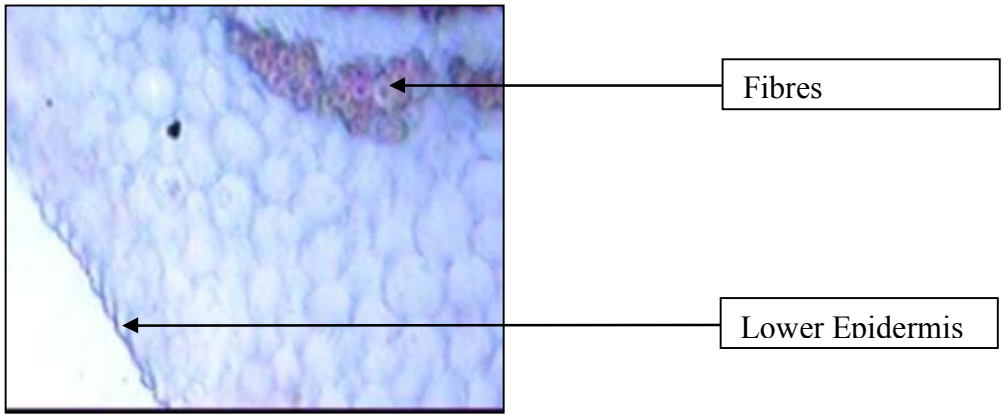


Fig 6.6 T.S of leaf, stained, at 500 x

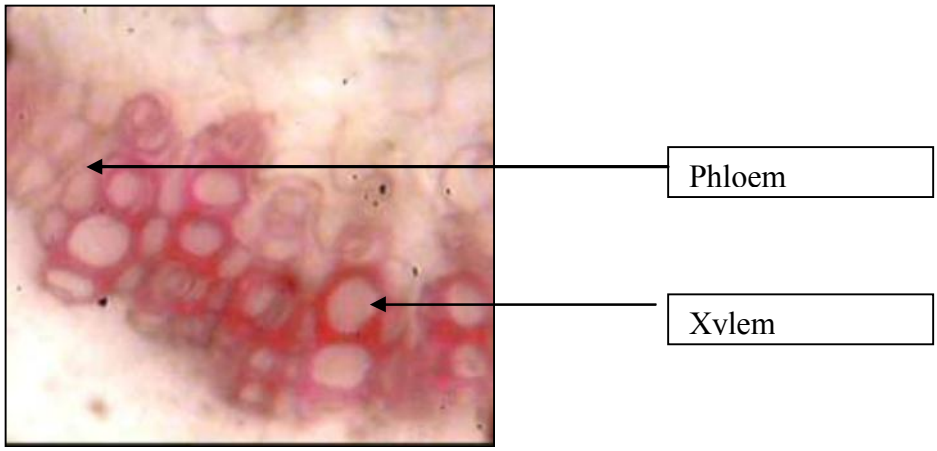


Fig 6.7 T.S of leaf, stained, showing xylem and phloem at 1800x

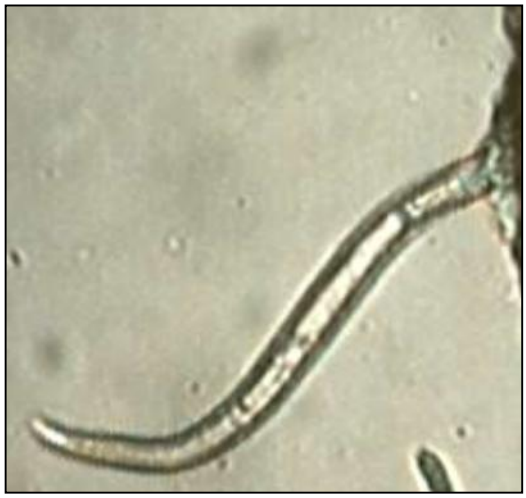


Fig 6.8 Trichome at 200x

Fig 6.9 Surface Preparation showing paracytic stomata at 200x

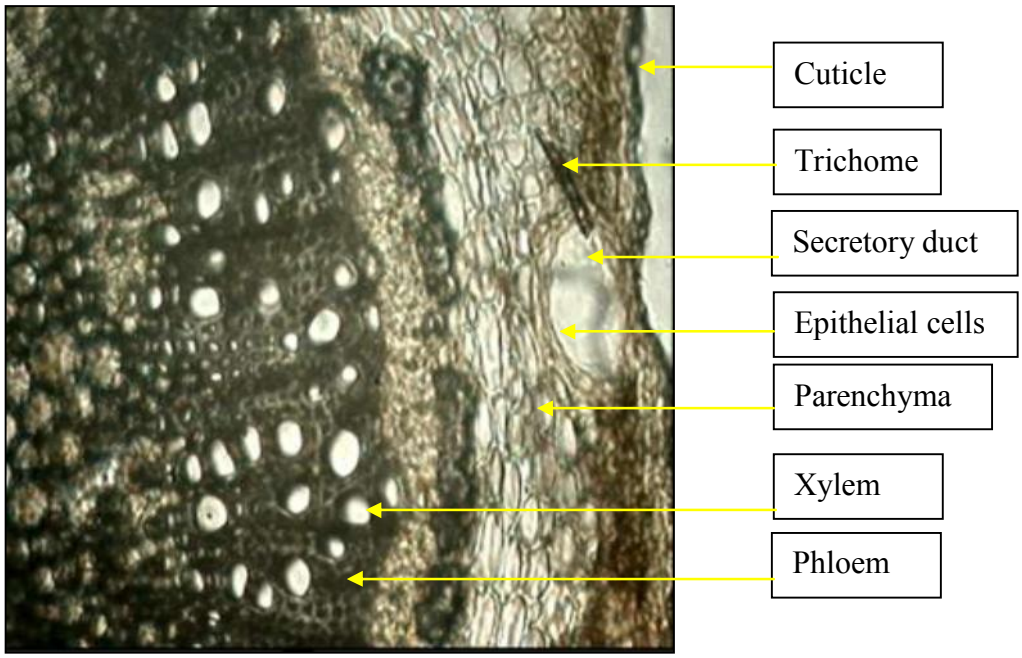


Fig 6.10 aT.S. of Petiole, unstained at 500 x

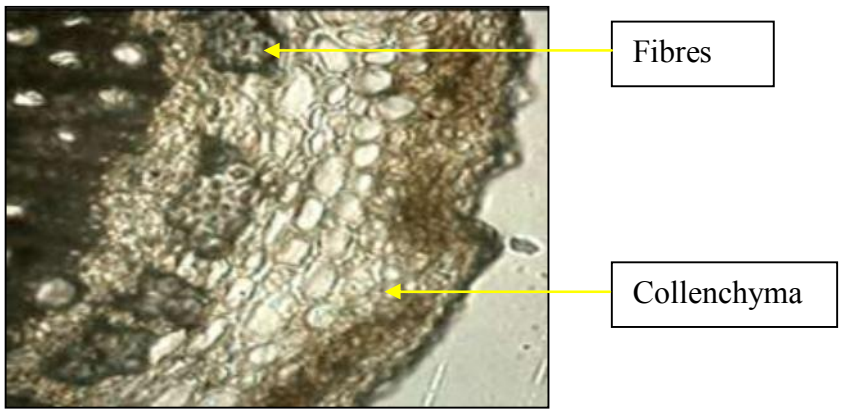


Fig 6.10 b T.S. of Petiole, unstained at 500 x

A.3 Physical profile

A.3.1 *Zingiber officinalis*

Table 6.1 Results of Physical Profile of *Z. officinalis*

Parameter	Obtained value (% w/w)	Reported value (% w/w) (Mukherjee, 2008)
Total Ash	4.3±0.5	<8.0
Acid Insoluble Ash	0.56±0.1	<1.0
Water Extractive Value	14.34±2	>14.0
Alcohol Extractive Value	9.95 ±2	>6.0
Loss on Drying	0.75 ±	-

A.3.2 *Boswellia serrata*

Table 6.2 Results of Physical Profile of *B. serrata*

Parameter	Obtained value (% w/w)	Reported value (%w/w)(Mukherjee, 2008)
Total Ash	6±1	<8.0
Acid Insoluble Ash	0.5±0.1	<1.0
Petroleum Ether Extractive Value	31.95± 2	20.0-35.0
Alcohol Extractive Value	37± 2	30.0-55.0
Loss on Drying	3.5±0.2	-

A.3.3 *Aegle Marmelos*

Table 6.3 Results of Physical Profile of *A. marmelos*

Parameter	Obtained value(% w/w)	Reported value (%w/w) (Siddqui *et al.*, 2010)
Total Ash	6.1±0.2	6.307
Acid Insoluble Ash	0.3±0.2	2.55
Water Extractive Value	2.3±0.3	2.93
Alcohol Extractive Value	8.06±0.2	8.66
Loss on Drying	0.63 ±0.1	0.74

A.4 Preliminary Phytochemical Screening

Table 6.4 Results of Preliminary Phytochemical Screening

Test	***Zingiber officinalis***	***Boswellia serrata***	***Aegle Marmelos***
Test for alkaloids	-	-	++
Test for carbohydrates	+++	+	++
Test for glycosides	-	-	-
Test for sterols	-	++	-
Test for amino acids and proteins	+	-	++
Test for saponins	+	-	+
Test for phenolic compounds and tannins	+	-	++
Test for flavonoids	_	_	+

+ Trace , ++ Intermediate , +++ More, ++++ Abundant, - Nil

A.5 Preparation of extracts

Table 6.5 Results Showing the Yield of Extract

Plant Name	**Solvent used**	**% yield w/w**
Zingiber officinalis	Water: Methanol (1:1)	10
Boswellia serrata	Methanol	35
Aegle Marmelos	Methanol	7.5

A.6 Result of TLC Analysis of Extracts

A.6.1 *Zingiber officinalis*

Solvent system: Hexane : Ether (4:6)

Spraying reagent: Vanillin sulphuric acid

No. of spots observed: 7

Solvent front: 4.8 cm

Rf of spots: 1) 0.27; 2) 0.5; 3) 0.56; 4)0.68;
5) 0.68; 6) 0.75; 7) 0.

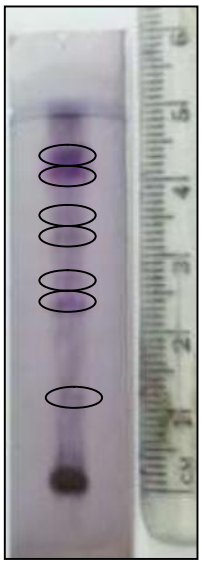


Fig 6.11a TLC of Ginger extract

A.6.2 *Boswellia serrata*

Solvent system: Hexane : Ethylacetate (7:3)

Spraying reagent: 10% sulphuric acid

No. of spots observed: 7

Solvent front: 4.8 cm

Rf of spots: 1) 0.17; 2) 0.27; 3) 0.39; 4) 0.56;
5) 0.66; 6) 0.82; 7) 0.94

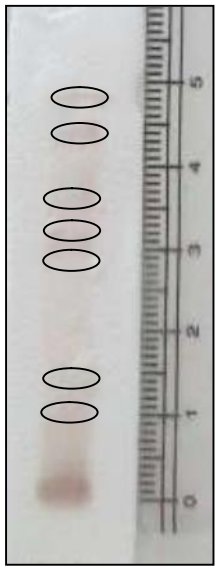


Fig 6.11b TLC of *B. serrata* extract

A.6.3 *Aegle marmelos*

Solvent system: Toluene : Ethylacetate : Formic acid. (7:3:0.1)

Detection: 366 nm

Spraying reagent: Anisaldehyde-sulphuric acid

No. of spots observed : 14

Solvent front: 6.2 cm

Rf of spots : 1) 0.14; 2) 0.21; 3) 0.28; 4)0.42;
5) 0.45; 6) 0.59; 7) 0.69; 8) 0.76
9) 0.88

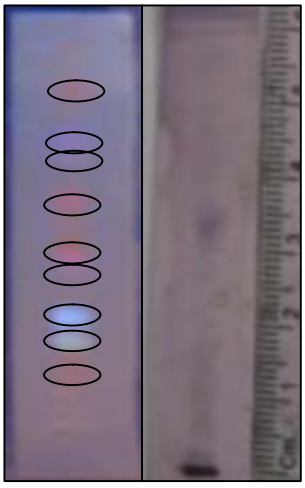


Fig 6.11c TLC of *A. marmelos* extract

B) DPPH Free Radical Scavenging Activity

The combined extracts of *Z. officinalis, B. serrata* and *A. marmelos* in the ratio of 1:1:1 was found to scavenge the free radical generated from methanolic solution of DPPH. The maximum inhibition (81.88%) was found to be concentration 200 µg/3ml. The Ginger extract shows more inhibition of free radicals (88.81%) as compared to combination. The concentration needed for 50% scavenging i.e. IC_{50} was found to be 13.06 µg/ 3ml for the combined extracts and 9.3 µg /3ml and 11.7µg/3ml of the Ginger and *A. marmelos* extracts respectively.

Table 6.6 Concentration of the solutions and % inhibition of free radicals

	% inhibition			
Concentration (µg/3ml)	***Combination (1:1:1)***	***Boswellia serrata***	***Zingiber officinalis***	***Aegle marmelos***
0.4	30.76±0.2	23.77±0.2	32.16±0.2	38.04±0.2
0.8	27.97±0.2	7.55±0.3	33.56±0.3	29.93±0.3
1.2	25.31±0.2	3.77±0.3	32.16±0.3	34.54±0.3
1.6	40±0.1	4.89±0.4	28.39±0.3	39.58±0.3
2	32.3±0.2	7.27±0.2	29.1±0.2	53.28±0.2
4	30.62±0.1	9.65±0.4	30.48±0.4	45.73±0.4
8	45.31±0.2	9.23±0.2	31.88±0.2	45.59±0.2
12	41.11±0.1	13.42±0.3	33.84±0.4	36.36±0.4
16	32.0±0.3	10.20±0.2	34.26±0.1	36.36±0.1
20	26.57±0.1	13.14±0.3	43.35±0.3	43.77±0.3
40	38.18±0.2	5.31±0.2	54.12±0.2	48.67±0.2
60	37.48±0.1	11.04±0.2	53.42±0.2	49.09±0.2
80	49.23±0.2	6.57±0.4	63.07±0.4	52.58±0.4
100	50.48±0.3	11.18±0.2	84.33±0.2	44.33±0.2
200	81.81±0.3	23.77±0.2	88.81±0.2	70.62±0.2

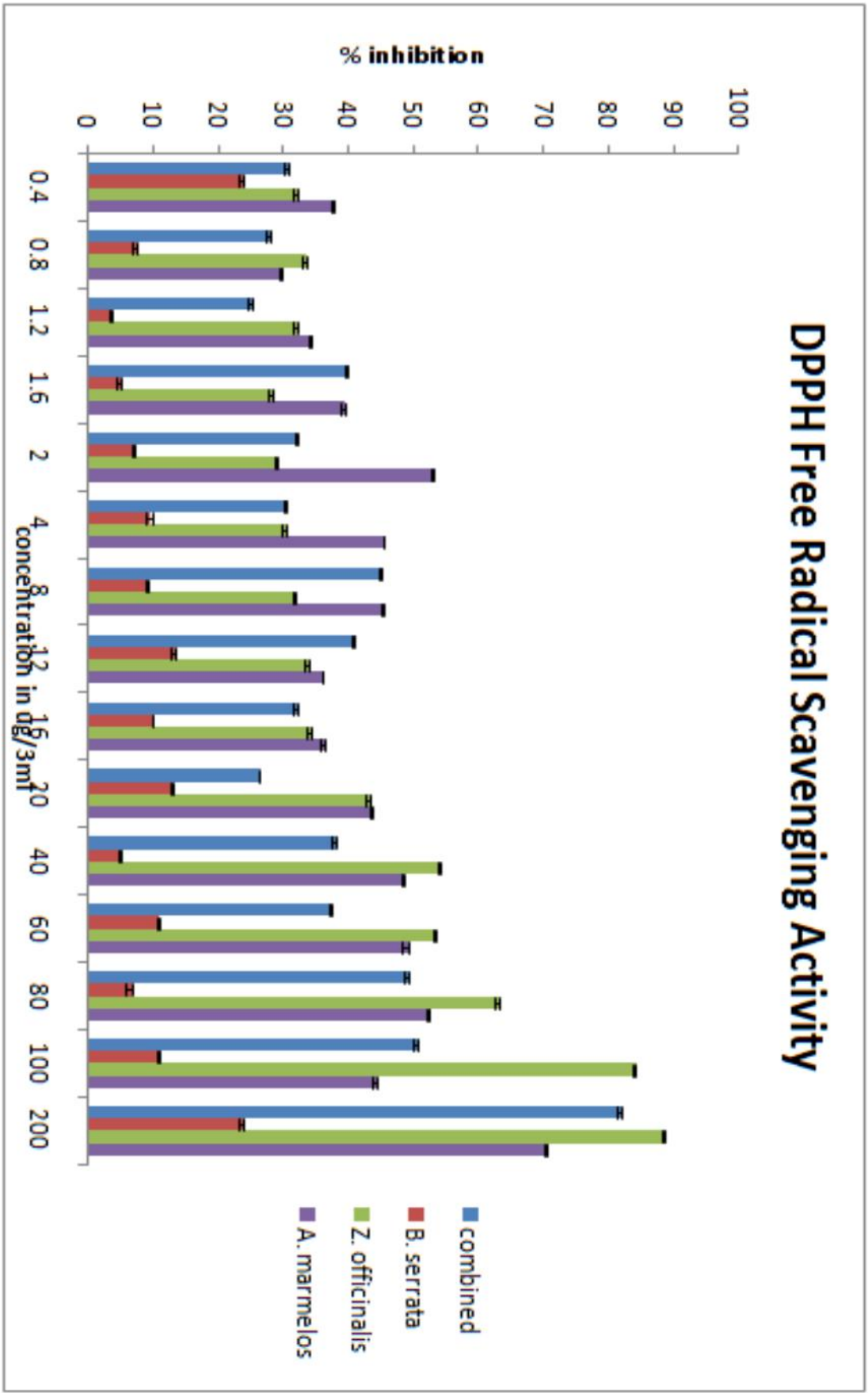


Fig 6.12 DPPH Free Radical Scavenging Activity

C) Optimization of Polymer Used in Gel

C.1 Results of Selection of Polymer

Table 6.7 Polymers used at various concentrations. X – Consistency not achieved, Y-consistency achieved.

Name of Polymer	Concentration	Result
Hydroxy propyl methyl cellulose 50 cps	5%	X
Polyethylene oxide 303	1%	X
Sodium caboxymethylcellulose	5%	X
Sodium alginate	5%	X
Carbopol 940	1.5%	Y
Carbopol 934	1%	Y
Solvent – water		

C.2 Results of Optimization of Polymer Concentration

Table 6.8 Gel prepared using different concentration of Carbopol and results of performed evaluation parameters, + good, - beyond the limit of instrument.

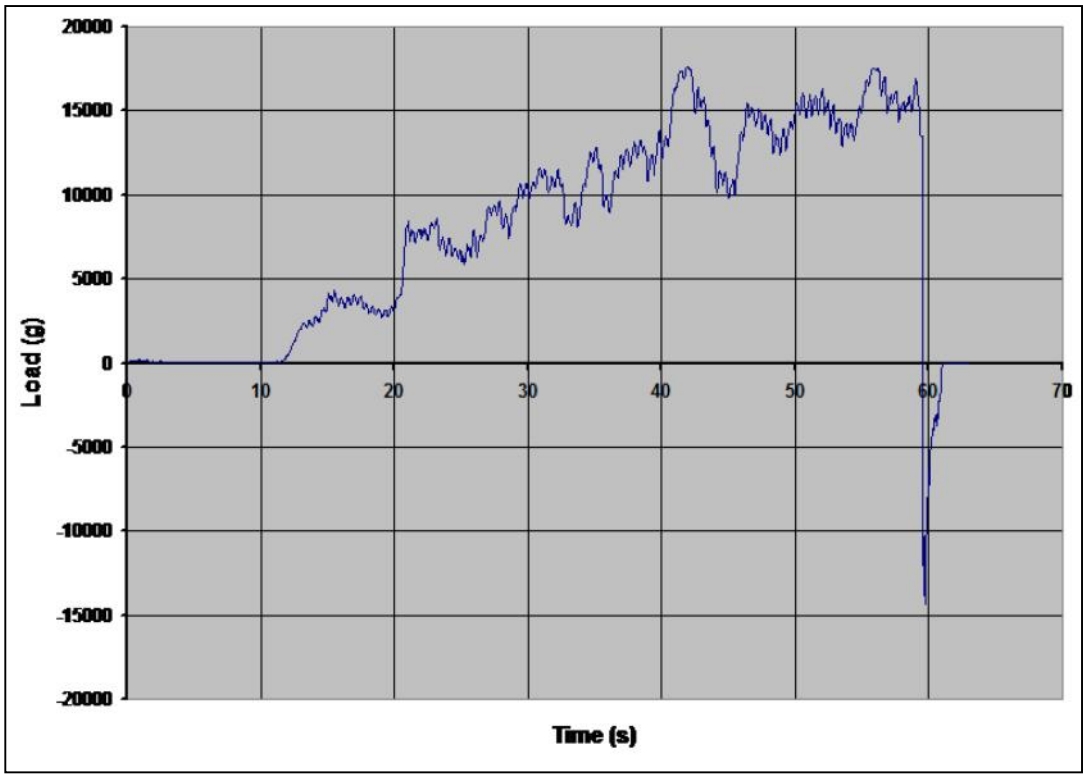
Gel with different concentration of polymer	Evaluation parameters			
	Viscosity (cps)	Torque (%)	Smoothness	Spreadability
0.4 %	7808	82.01	+	+
0.5%	8345	64	+	+
1%	9004	40	+	+
1.5%	-	-	+	+
Standard (piroxicam)	5077	23.4	+	+

C.3 Result of gel extrusion test.

Table 6.9 Evaluation of adhesive force, peak load, average peak adhesiveness, mean load and final load of gel prepared with the different concentrations of carbopol and its comparison with the standard (piroxicam) marketed preparation.

Concentration	Adhesive force	Peak load	Average peak (g)	Adhesiveness (gs)	Mean load	Final load
0.4%	1167.00	17577.00	1154.59	-7644.15	49.31	-1035.00
0.5%	15274.00	14975.00	775.11	-11254.95	134.15	14562.00
1%	16101.00	16242.00	1617.72	-13565.33	173.04	15818.00
1.5%	16975.00	15244.00	3677.53	-14223.43	134.69	-9148.00
Standard	14360.00	17601.00	1600.63	-8229.22	119.23	15879.63

C.3.1 Graphs



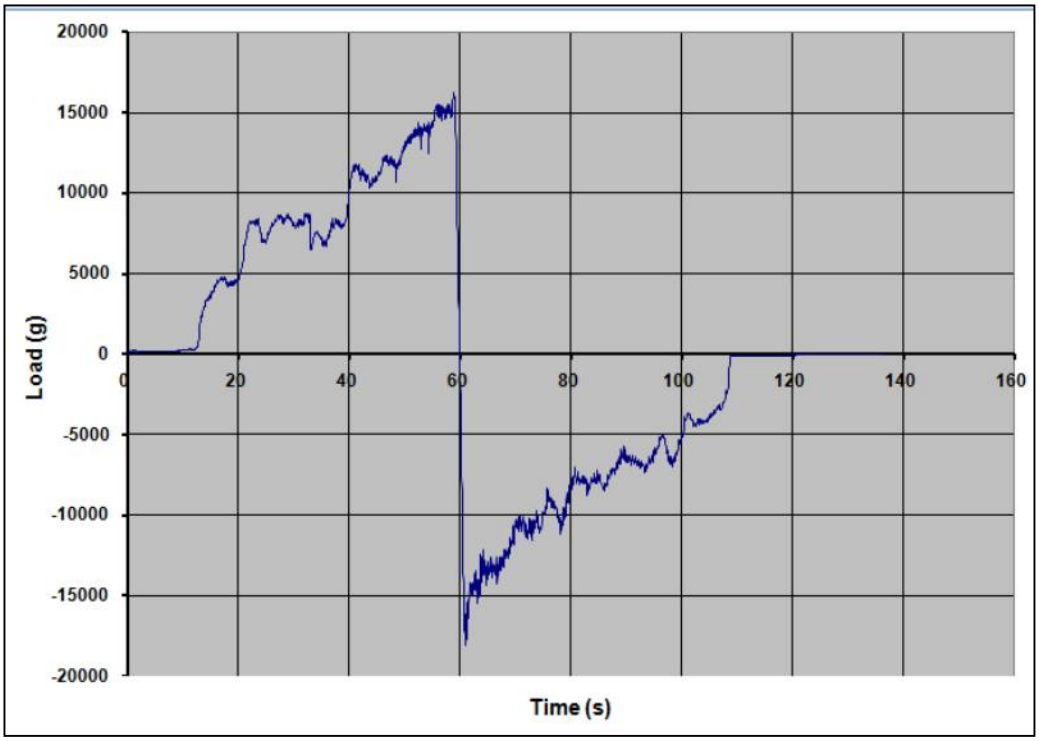


Fig 6.13 Graph of Load vs. time - Standard

Fig 6.14 Graph of Load vs. time – 1% polymer

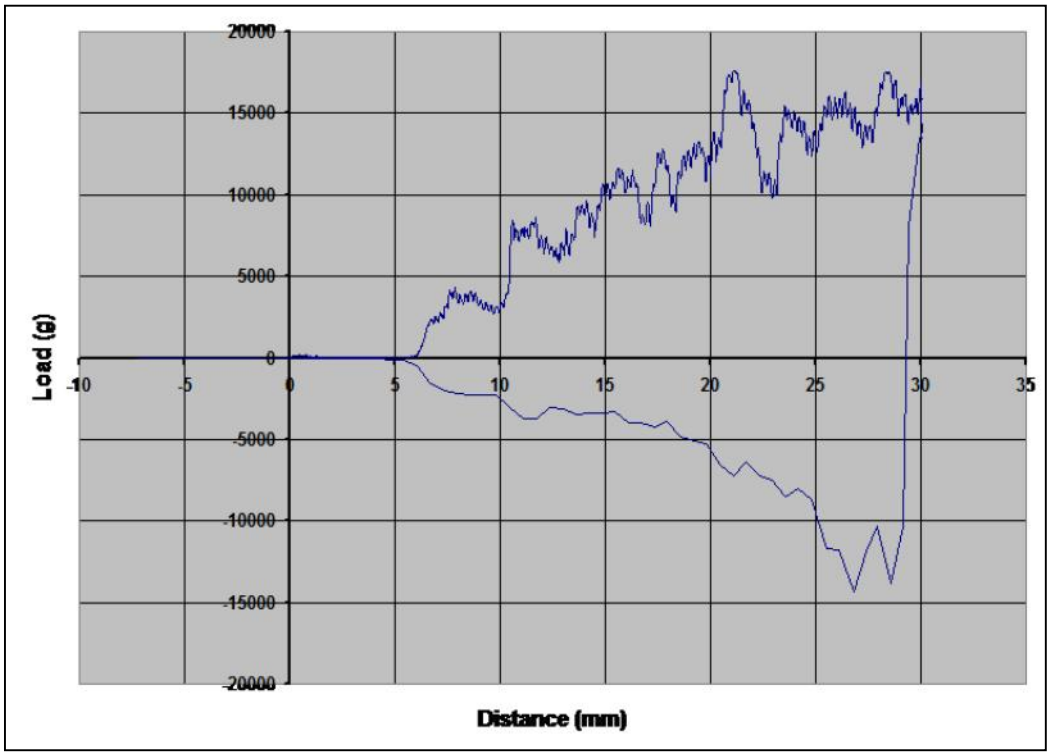


Fig 6.15 Graph of Load vs. Distance - Standard

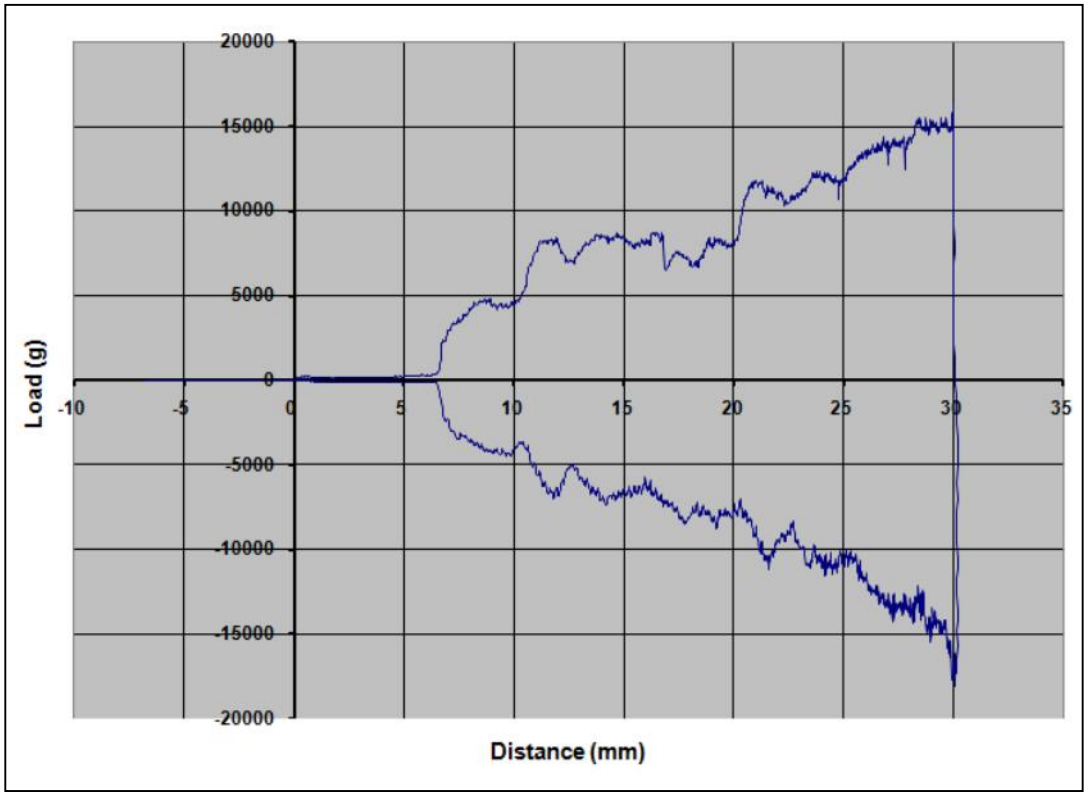


Fig 6.16 Load vs. Distance – 1% polymer

C.4 Composition for the gel

Table 6.10 Composition of the gel

Phytoconstituents	0.5 gm of extract of *Zingiber officinalis* 0.5 gm of extract of *Boswellia serrata* 0.5 gm of extract of *Aegle marmelos*
Polymer	Carbopol 934 (1%)
Solvent	Water (30 ml)
Solvent to dissolve extracts	*Zingiber officinalis*- isopropyl alcohol+ water(1+1 ml) *Boswellia serrata* - isopropyl alcohol (2 ml) *Aegle marmelos* - isopropyl Alcohol (2 ml)
pH enhancer	Triethanolamine (q.s)
Preservative	Methyl paraben (0.03 gm)

D) Carrageenan induced paw edema

The result shows that the formulation containing combined extracts shows maximum percentage inhibition 74.7 % of the paw volume. The reduction in the paw volume was found to be significant with respect to control groups.

Table 6.11 Results of Carrageenan Induced Paw Edema

		Paw volume at various time intervals after carrageenan injection				
Groups / Time	**No. of Animals**	**0 hr**	**1 hr**	**2 hr**	**3hr**	**4 hr**
Control – Base treated	**5**	1.3±.02	1.5± .01	1.53± .01	1.55±.02	1.56± .02
Formulation treated	**5**	1.23 *± .01	1.38* ± .01 (21)	1.32* ± .01 (63.2)	1.32*±.01 (69.3)	1.31* ± .01 (74.7)
***Z. officinalis* gel- treated**	**5**	1.07± .01	1.2± .02 (18.9)	1.18± .01 (35)	1.17±.02 (48)	1.16 ±.01 (55.6)
***B. Serrata* gel-treated**	**5**	1.3 ±.01	1.43± .01 (34.6)	1.45± .02 (35)	1.39±.01 (58.3)	1.39± .01 (64)
***A. marmelos* gel-treated**	**5**	1.21± .02	1.36± .01 (19.6)	1.33± .01 (44)	1.32±.02 (46.3)	1.32± .01 (47.4)
Standard	**5**	1.14*± .01	1.29*± .01 (17.7)	1.29*±.01 (68.2)	1.19*±.02 (71.4)	1.18* ± .01 (80)

The result are shown in mean volume ± SEM, () percentage reduction. * p<0.05

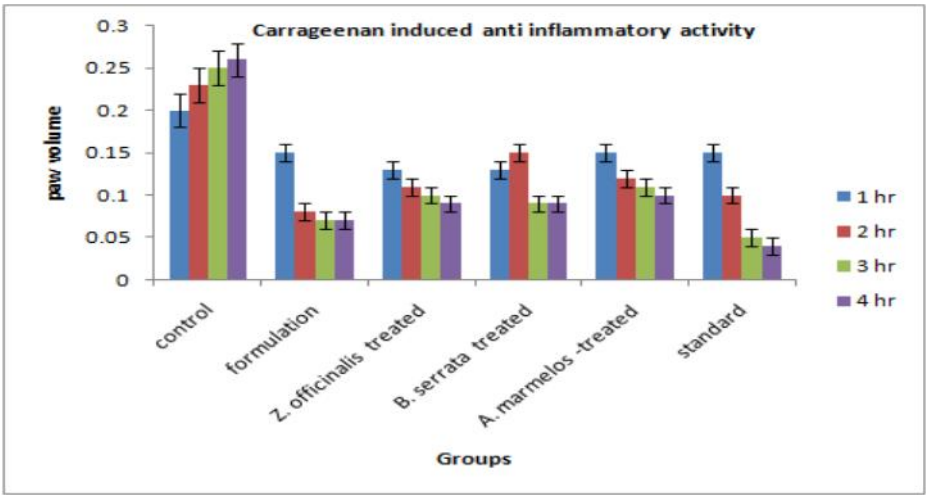


Fig 6.17 Carrageenan induced paw edema activity, showing the comparison of the reduction in paw volume in ml at various interval of time.

E) Adjuvant Induced Developing Arthritis in Rats

The result shows that on 13th day after the injection of CFA maximum inhibition was seen on the last day. The percentage inhibition by the formulation was found to be 67.6% and for the standard it was found to be 75%. The results were significant with respect to control.

Table 6.12 Result of Adjuvant Induced Developing Arthritis Activity

		Paw volume at various days after immunization with freund's adjuvant.						
Groups / Days	**No. of animals**	**1**	**3**	**5**	**7**	**9**	**11**	**13**
Control – Base treated	**6**	1.545 ± 0.1	2.58 ± 0.2	2.72 ± 0.2	2.45 ± 0.1	2.11 ± 0.05	2.54 ± 0.2	2.4± 0.1
Formulation treated	**6**	1.69 * ± 0.04	2.39* ± 0.2 (10)	2.42* ± 0.2 (30.2)	2.34* ± 0.2 (63.4)	2.005* ± 0.05 (42.6)	1.99* ± 0.1 (61.69)	1.92* ± 0.08 (67.6)
Standard (piroxicam gel 0.5 %)	**6**	1.31 * ± 0.1	2.05* ± 0.2 (10)	2.12* ±0.2 (30.2)	1.74* ± 0.1 (63.4)	1.54* ± 0.05 (42.6)	1.54* ± 0.2 (61.19)	1.45* ± 0.1 (75)

The result are shown in mean volume ± SEM, () percentage reduction. * p<0.05

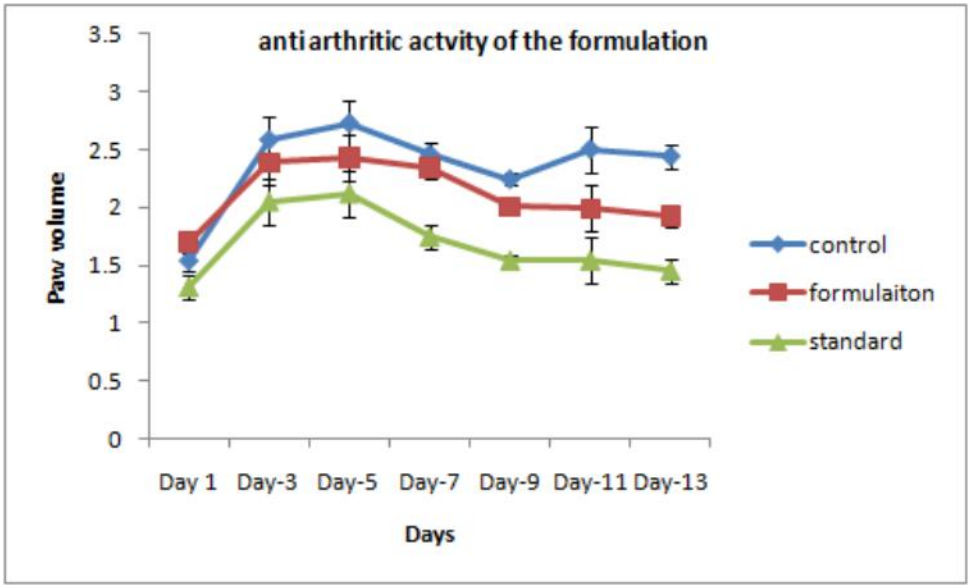


Fig 6.18 Comparison of the paw volume between the three groups on alternate days after starting the experiment

F) Estimation of 6-Gingerol by HPTLC

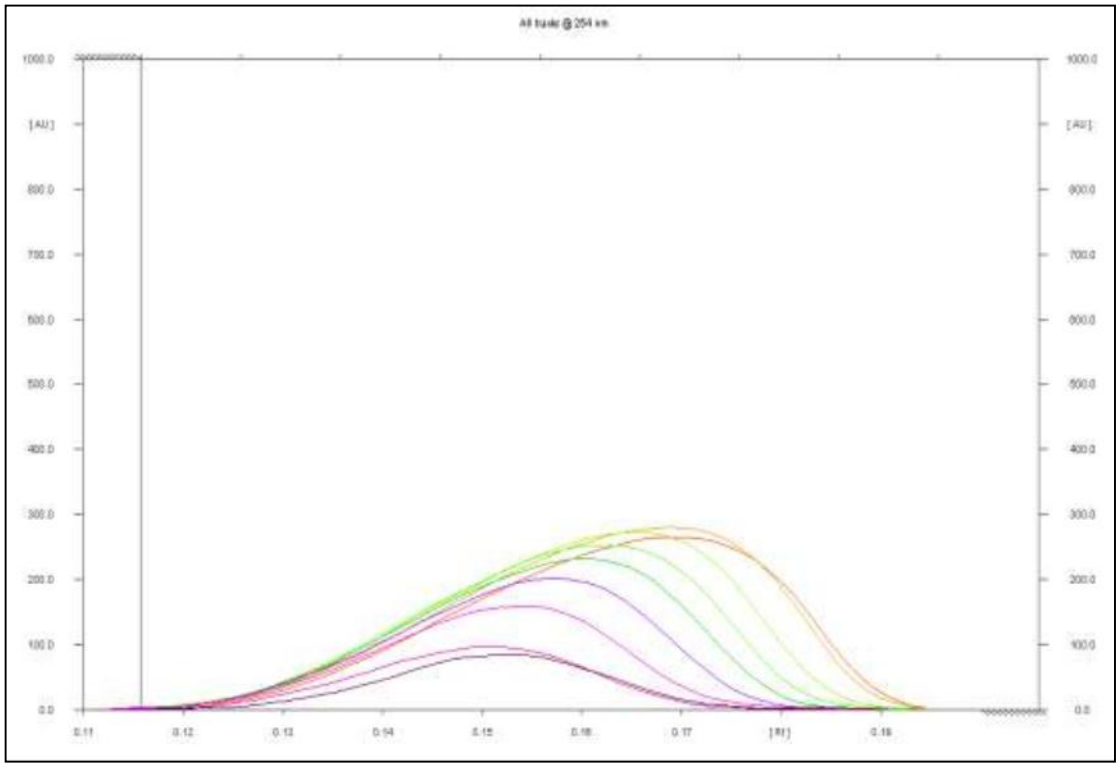


Fig 6.19 Overlay Spectra of 6-Gingerol

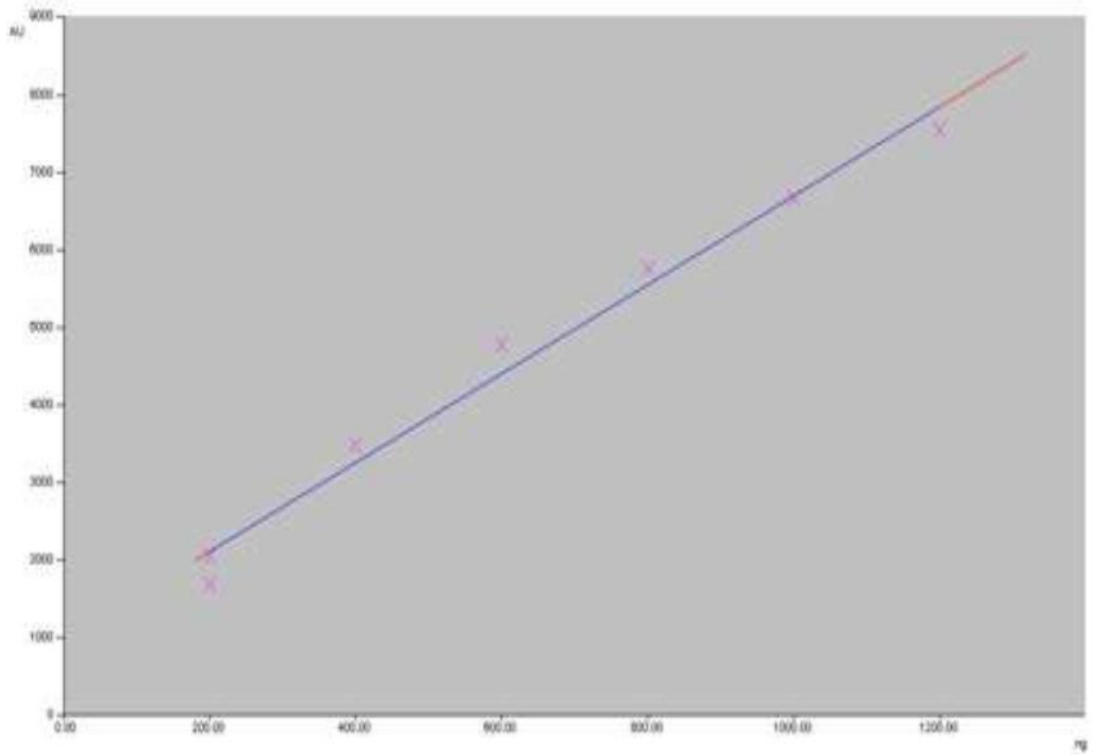


Fig 6.20 Calibration curve of 6-Gingerol

R^2 value = 0.99183, Equation : Y =956.2503+ 5.6066*X

Area under Curve = 2661.67 for 5 µl.

Rf : 0.18; Scanned at 254 nm

The amount of 6- Gingerol calculated with the help of calibration curve in 1 gm of gel was found to be 0.0059 % w/w.

Table 6.13 Concentration of each spot and its area under curve

Spots no.	Concentration (ng)	Area under peak
1	200	1978.76
2	400	345805.
3	600	4654.05
6	800	5684.25
7	1000	6567.60
8	1200	7349.28

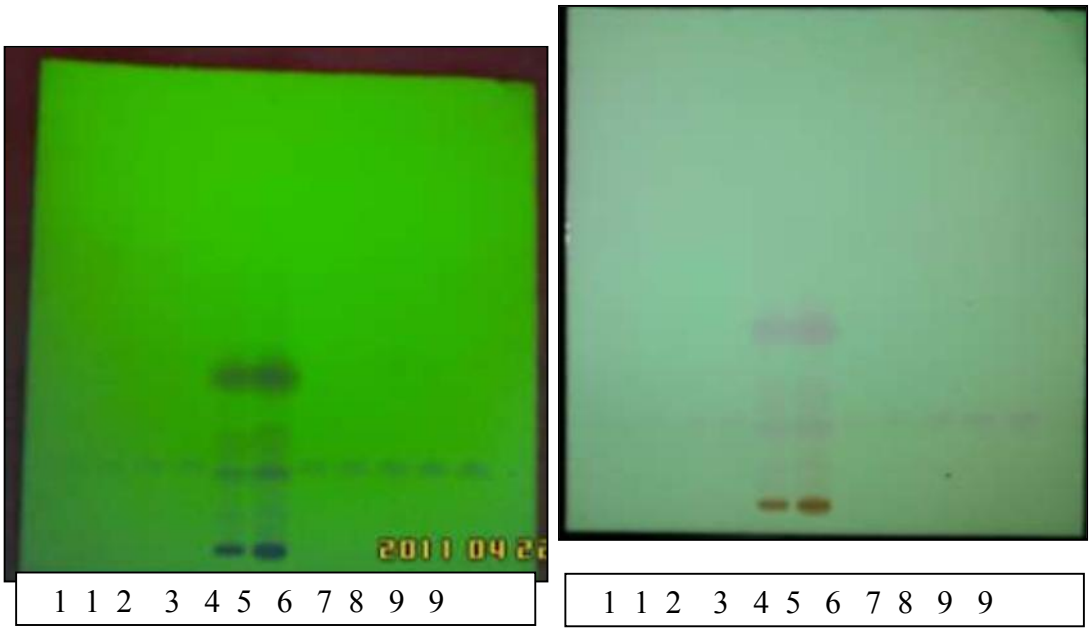


Fig 6.21 Precoated Plate of HPTLC of 6- Gingerol. Spot 1- 200 ng; 2- 400ng; 3- 600 ng 6-800ng 7-1000; 8-1200; 9-1400 of Gingerol; 4 and 5- Test sample

Chapter 7

Discussion

7. DISCUSSION

Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder in which the immune system which normally protects the body by fighting infections and diseases, instead targets the body. RA is different from other types of arthritis such as osteoarthritis, a wear-and-tear condition that commonly occurs as people age. In RA, the immune system attacks the tissues that line the joints, causing pain, swelling, and stiffness in the joints and affecting their ability to work properly. Over time, RA may damage bone and cartilage within the joints, weaken muscles and tendons that support the joints, and lead to joint destruction. Rheumatoid arthritis is characterized by persistent synovitis, systemic inflammation, and auto antibodies**.** RA reduces a patient's lifespan by about 10 years if only the symptoms are treated. It is also associated with the co-morbidities: Increase the risk of heart disease (Weaver, 2004), permanent joint damage which may lead to loss of function. (Callahan and Pincus, 1995).

Plant extracts have been used for centuries as a popular mode of treatment for several health disorders. Our target was to use the different extracts in the combined form in a gel formulation. Gels consist of a solid three-dimensional network that spans the volume of a liquid medium and ensnares it through surface tension effects. Hydrogel is a network of polymer chains that are hydrophilic, found as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent as they can contain over 99% water and possess a degree of flexibility, due to their significant water content. The polymer carbopol provides sustained release of drug.

The dried powder of *Z. officinalis*, gum resin of *B. serrata* and the leaves of *A. marmelos* were subjected to macroscopical examination and observations were recorded. The proper examination was carried out under sun light and artificial source similar to day light. The microscopic study of the leaf of *A. marmelos* showed that midrib is slightly pronounced towards lower surface. It showed single layered epidermis covered with thick cuticle (Fig 6.5), cells of upper epidermis are bigger in comparison to lower surface. Ground tissue parenchymatous, vascular bundle consists of radially arranged xylem (endarch), (Fig 6.4), and encircled by phloem (Fig 6.7). Rare trichomes, found mainly on lower surface of mid-rib region and are unicellular

(Fig6.8), stomata are paracytic as it is shown in surface preparation of the leaf (Fig 6.9). The unstained transverse section of the petiole showed trichomes rarely like midrib; single layered upper epidermis composed of polygonal tubular cells, covered with thin cuticle; shows followed by 3 - 5 layers of collenchymatous hypodermis; parenchymatous ground tissue containing large, circular secretory duct, vascular bundle were radially arranged, encircled by phloem and pith broad and parenchymatous (Fig 6.10).

All the results of physical profile were within the pharmacopeial limits (Table 6.1, Table 6.2 and Table 6.3). The results of preliminary phytochemical screening in Table 6.4 showed the presence of carbohydrates, phenolic compounds and saponins in *Z. officinalis. B. serrata* gives positive tests for sterols and phenolic compounds and tannins. A. marmelos showed the absence of sterols and glycosides and presence of flavanoids, alkaloids and carbohydrates. The % yield of hydroalcoholic extract of *Z. officinals,* methanolic extract of *B. serrata* and methnolic extract of *A. marmelos* were found to be 10%, 35%and 7.5% respectively (Table 6.5). The TLC of Ginger extract was carried out using solvent system Hexane : Ether (4:6) and spraying reagent was vanillin sulphuric acid. 7 spots were observed in TLC (Fig 6.11a). The TLC of methanolic extract of salai guggal was carried out using solvent system Hexane : Ethylacetate (7:3) and spraying reagent was 10% sulphuric acid. Again 7 spots were observed in TLC (Fig 6.11b). The TLC of *A. marmelos* extract was carried out using solvent system Toluene : Ethylacetate and Formic acid (7:3:0.1), UV light at 366nm at and spraying reagent was anisaldehyde sulphuric acid. 9 spots were observed in 366nm and 1 spot observed after derivatization, (Fig 6.11 c).

In healthy organisms, production of reactive oxygen species and reactive nitrogen species is approximately balanced by antioxidant defence systems. Increased oxidative stress and/or defective antioxidant status contribute to the pathology of rheumatoid arthritis (Mahajan and Tandon, 2004). It is well known that the antioxidant activity of plant extracts containing polyphenolic components are due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests used to prove the ability of the components of the extracts to act donate hydrogen atoms. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical because of its unpaired electron delocalization over the whole molecule. The delocalization causes a deep violet color with substrate at wavelength

517 nm. When a solution of DPPH is mixed with a substrate acting as a hydrogen atom donor, a stable nonradical form of DPPH is obtained with simultaneous change of the violet color to pale yellow (Stoilova et al., 2007). The results of antioxidant activity were given in the Table 6.6. The combination of three extracts (1:1:1) showed maximum inhibition of 81.8 % at concentration 200 µg / 3ml and its IC_{50} was found to be 13.06 µg / 3ml. The percentage inhibition *of Z. officinalis* extract*, B. serrata* extract and *A. marmelos* extract were found to be 88.8%, 70.6% and 23.7% respectively. The results showed that the inhibition of free radicals by the Ginger extract was more than that of the combined extract which indicates that there was no synergy present in the combination against free radical scavenging. The IC_{50} value of *Z. officinalis* and *A. marmelos* extract were found to be 9.3 µg/3ml and 11.7 µg/3ml respectively. In preliminary phytochemical screening, both Ginger and *A.marmelos* had shown the presence of phenolic and tannin compounds but salai guggal had shown no presence of the phenolic compounds which lead us to the inference that DPPH free radical scavenging activity may be due to Ginger and *A. marmelos* extract and this activity may be helpful in the treatment of RA as many reactive oxygen species are involved in the pathogenesis of RA.

Gels are defined as per USP as semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. The Table 6.7 showed that carbopol 934 used in 1 % concentration attained the required consistency for the gel. Other polymers, hydroxypropyl methyl cellulose 50 cps, polyethylene 303, sodium carboxymethyl cellulose, sodium alginate even in the high concentration were not able to achieve consistency. The result for the evaluation parameters carried out (Table 6.8) to optimize the concentration of the polymer carbopol 934, showed all the gels were having good spreadability and smoothness. The viscosity at 100 rpm of the gel containing 1.5% polymer was not measurable by the instrument Brookfield viscometer.

Results of gel extrusion test by texture analyzer (Table 6.9) showed that 1 % gel had required more adhesive force, 16101 as compared to that of standard 14360. The adhesiveness of 1% polymer was also found to be higher -13565.33 than that of standard, -8229. Increase in adhesivness is good for the gel as it will not be wiped off easily (Fig 6.13 to Fig 6.16). As compared to other concentration of the polymer 1%

concentraion was good for the formulation of the gel. The final composition of the gel is given in the Table 6.10.

Carrageenan paw inflammation is one of the most commonly used models for the investigation of new anti-inflammatory agents (Villar *et al.,* 1987). The elevation of the paw volume in the first hour is due to the effect of histamine and serotonin on the vascular permeability. Inflammation gradually increases and attains peak within 3 to 4 h. This second phase could be due to the liberation and over production of prostaglandins and kinins in paw tissue, which accompanies leukocyte migration. (Singh *et al.,* 2008). The results of carregeenan induced paw edema were given in the Table 6.11 showed that formulation containing combined extract in the ratio 1:1:1 had maximum inhibition 74.7% as compared to 55.6%, 64% and 47.4% of *Z. officinalis* gel, *B.serrata* gel *and A. marmelos* gel respectively. The % inhibition by standard piroxicam gel (0.5%) was 80%. Ginger has been proved to be non selective COX inhibitior but unable to inhibit TNFα. (Lantz et al., 2007). The extract of Boswellia has been proved as anti-inflammatory through the inhibition of TNFα, IL-1β, NO and MAP kinases (Gayatri et al., 2007). From the result it can be predicted that the synergy obtained in the anti-inflammatory activity may be due to the whole arachidonic acid (AA) cascade and preliminary factors involved for AA cascade are inhibited. The 11-keto-β-boswellic acid is a lipoxygenase inhibitor and presence of compounds in *A. marmelos* extract may also enhance the activity.

In arthritis, joint swelling and pain are the most commonly co-existing symptoms. An ideal therapeutic agent should at least possess anti-inflammatory and analgesic property. As the application was topical so only the inhibition of paw volume by the gel was carried out. The results of the inhibition of the paw volume were given in the Table 6.12 show that the inhibition of paw volume by the formulation containing three extract is 67.6% whereas the inhibition by the standard is 75%. The primary action of the freund's adjuvant results in the production of interleukins and TNFα. The production of IL-6 results in the generation of autoimmunobodies which in later phase causes joint destruction. The release of chemokines and cytokines lead to the generation of AA cascade and production of prostaglandins which further exaggerates the condition. The *B. serrata* extract of has been proved for the immunomodulatory activity, as well as for the inhibition of the TNFα, interleukins and lipooxygenase.

Ginger is reported for nonselective COX inhibitor which leads to the inhibition of prostaglandins. The methanolic extract of *A. marmelos* has reported for its analgesic and anti-inflammatory activity like that salai guggal.

The estimation of 6-gingerol in the formulation was carried out using HPTLC. The standard solution of 6 – gingerol was applied in the range of 200 – 1400 µg to 10 x 10 plate of silica gel F_{60} 254. The overlay spectra of 6-gingerol and the calibration curve were showed in the Fig 6.19 and 6.20. the linearity was obtained between 200 – 1200 µg. The percentage of 6-gingerol in the gel was found to .005% w/w in 1gm of gel.

Chapter 8

Conclusion

8. CONCLUSION

Indian herbal medicines are increasingly becoming popular world wide, and there market is increasing day by day. Using polymer carbopol we have formulated a satisfactory gel containing extracts of three different herbal drugs in the same ratio. Based on our study we conclude that the formulation shows significant activity against inflammation and the combination of the drugs have shown synergistic effect when they are given through topical application. But the same formulation did not show synergistic effect for antioxidant activity although the combination possesses good antioxidant activity. The formulation has also shown significant inhibition of paw volume in adjuvant induced developing arthritis in rats. Further studies are required to be carried out like the bioavailabilty of the 6-gingerol and 11-keto-β-boswellic acid. It is also required to study the effect of the combination in another ratio and/or in another dosage form is significant or not so that the formulation may be useful for arthritis after further clinical trials.

Chapter 9

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8. REFERENCES

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Date: 12/04/2011

CERTIFICATE

This is to certify that the specimens of the plants provided by following students of Institute of Pharmacy, Nirma University, Ahmedabad are authenticated by me.

Sr. No.	Student Name	Plant Name
1	Kavita Mehta	*Typha angustata*
2	Naeem Devla	*Shorea robusta*
3	Mrudul Kansara	*Myristica fragrance*
4	Dhruv Rawal	*Boswellia serrata* *Commiphora wightii*
5	Nidhi Jasani	*Lagenaria siceraria*
6	Hiral Kataria	*Gmelina arborea*
7	Neha Modi	*Aegle marmelos* *Zingiber officinale*
8	Megha Kapoor	*Ficus religiosa*
9	Ratnesh Joshi	*Hemidesmus indicus*
10	Urvi Viroja	*Achyranthes aspera*
11	Ujjawal Kansara	*Butea monosperma*

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PUBLICATIONS AND PRESENTATIONS

Presentations:

1. Modi, N., Mehta, K., Acharya, N., Acharya, S., Tripathi. N., Kumar, V., "Formulation and Evaluation of Mahayograj guggul vati and its comparison with marketed formulation" at AYUSH sponsored National Seminar on "Current Trends And recent Advancements in Herbal Drugs" held on 22nd Jan 2011 KBIPER Gandhinagar (Gujarat).
2. Modi, N., Mehta, K., Acharya, N., Acharya, S., Kumar, V., "Natural compounds as a guide to lead new drug discovery for cancer", GUJCOST Sponsored National Seminar on "Development of Rational Botanical Formulations", held on 5th Dec. 2010 at Kalol Institute of Pharmacy, Kalol, Gandhinagar (Gujarat).
3. Modi, N., Mehta, K., Acharya, N., Acharya, S., Kumar, V., "Phytoremediation: A measure to restore environmental balance", GUJCOST Sponsored National Seminar on "Development of Rational Botanical Formulations", held on 5th Dec. 2010 at Kalol Institute of Pharmacy, Kalol, Gandhinagar (Gujarat).

Neha Modi **Prof. (Dr.) Vimal Kumar**