

Evaluation of Antiarthritic Activity of Cordyline Fruticosa in Arthritic Rat Model

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Abstract

A persistent inflammatory process that causes joint injury and deformity, dysfunction, and sometimes even death is a hallmark of rheumatoid arthritis, a systemic Autoimmune disorder. Rheumatoid arthritis (RA) is a systemic, autoimmune multisystem illness that manifests as Pain, synovial membrane inflammation, peripheral joint inflammation, morning stiffness, and tissue deterioration, and reduced joint range of motion. Cordyline fruticosa L also known as cordyline terminalis L belonging from family Agavaceae is commonly referred to as "good luck plant" and "Ti plant. Several different chemicals were found in C. fruticosa, including cholestane glycosides, polyphenols, flavonoids, glucosylfructan, tannins, alkaloids, steroidal saponins, and phytosterols, according to a phytochemical analysis. Pharmacological studies show that the plant has antimicrobial, antioxidative, and therapeutic properties related to stomach cancer. Following an examination of the literature, plant material was selected, collected, and authenticated. The collected plant material was dried and extracted with methanol as the solvent. The obtained methanolic extract underwent phytochemical study and GC-MS analysis to identify bioactive components. Pharmacological evaluations were carried out both in vitro and in vivo. Protein denaturation assays were used to determine in-vitro anti-inflammatory effects, which included the inhibition of egg albumin and bovine serum albumin. The in vivo anti-arthritic activity was assessed utilizing the Complete Freund's Adjuvant induced arthritis and formaldehyde-induced arthritis models. Various physical, biochemical, haematological, and histological characteristics were assessed, and the results were statistically analysed. The potency of cordyline fruticosa extract in methanol in formaldehyde induced arthritis as well as in complete Freund's adjuvant rats has shown its excellent anti-arthritic activity. It has shown remarkable reduction in joint inflammation, arthritic scores, and paw edema in a dose-dependent fashion. These findings support the use of Cordyline fruticosa to treat inflammatory conditions and show that it can be used as a safe and effective alternative to arthritis treatment.

CHAPTER – 1

Introduction

1. INTRODUCTION:

Rheumatoid arthritis (RA) is a systemic autoimmune disorder marked with a persistent inflammatory process that results in joint destruction, deformity, and dysfunction capacity, and sometimes even demise. This disorder affects 0.5 - 2% of persons, and is more prevalent in smokers, women and those with a history of the illness.¹ The symptoms of rheumatoid disease, a systemic Immune mediated multisystem illness, include discomfort, inflammation of the synovial membrane, Inflammatory reaction of the peripheral joints, Joint stiffness in the morning, degradation of Articular structures and limited range of motion in joints.¹⁵ In addition to Reactive oxygen intermediates, eicosanoids like leukotrienes, prostaglandins, and cytokines are some of proinflammatory chemicals released by macrophages that cause RA, a disease that can still cause severe functional impairments in addition to cartilage degradation.¹

Through enzyme inhibition such as COX and LOX and regulation of mediators released from immune cells and macrophages, chronic inflammatory diseases can modify the pathway of arachidonic acid metabolism. It can also affect blood arteries, coronary arteries, respiratory tract, and skeletal muscles, but mostly targets the joints, resulting in an expanding, inflammatory, or non-suppurative synovitis that frequently develops to articular cartilage loss and joint stiffness.³



Fig no.1 Rheumatoid arthritis (RA)

It primarily affects the joints, causing an expansive, inflammatory, or nonsuppurative synovitis that often leads to articular cartilage loss and joint stiffness. It can also impact the blood vessels, coronary arteries, respiratory system, and skeletal muscles. One of the most enduring immune disorders in the world is rheumatoid arthritis. Although the illness can begin at any age between 20 and 80, it usually manifests between the ages 40 to 50. Women are three times as likely than men to be impacted.⁴

Even while a number of categories, including NSAIDs, steroidal anti-inflammatory medications, and immunosuppressants, are already in use. Their use is limited by possible adverse effects. The creation of novel antiarthritic medications that are safe, effective, and less toxic is currently a major concern. Therefore, it is necessary to look for more naturally occurring substitutes so that their therapeutic benefits can be evaluated and increased.⁵

Such agents aim for lessening Painful inflammation, prevent and lessen Joint destruction, maintain structure and functionality in the joints, also enhance individual's feeling for wellbeing. These synthetic medications frequently have negative side effects. gastrointestinal discomfort, which encompasses drug addiction, cardiovascular issues, etc. However, because of Their low efficacy, delayed beginning of action, toxicity, high cost, long-term side effects, and the emergence of symptoms after withdrawal, these medications are not recommended for long-term treatments.⁶

Numerous inflammatory mediators are crucial for bone degradation during the start and course of rheumatoid arthritis. $\text{TNF-}\alpha$, interleukin- 1β , interleukin-6, nitric oxide (NO), prostaglandins, reactive oxygen species (ROS), platelet activating factor, leukotrienes, and enzymes (lipoxygenases, cyclooxygenases (COX-1 and COX-2), and phospholipases) that cause blockage and Synovial inflammation⁷

The primary signs of arthritis disorder (RA), a prolonged degenerative joint disorder, are Chronic proliferative synovitis, inflammatory cell infiltration of joint synovial tissue, and cartilage degradation. If it quickly develops becoming a multisystem inflammation with irreparable joints degradation, chance for

death may increase.⁸ The characteristics of RA include deformity, loss of bone, joint space, and function, as well as ongoing, irreversible destruction to the synovium lined joints. The breakdown of extracellular matrix, which results in the usual degeneration of bone, cartilage, ligaments, and tendons, is one of the main characteristics of RA⁹

In conditions of inflammatory arthritis like rheumatoid arthritis (RA), one of the most common types of connective tissue illness, Articular synovial membrane is harmed from variety of plasma cells and lymphocytes. Inflammatory pannus is the resultant thickening, congestion, and swelling of the membrane. The inflammatory pannus itself degrades articular cartilage, subchondral bone, and periarticular ligaments and tendons. Progressive joint deterioration is followed by fibrosis, increasing deformity, and restricted movement' morning stiffness, shoulder, neck, and pelvic girdle pain, feverish immobility, fatigue and weakness¹⁰

Pannus formation, fibrosis, synovial cell proliferation, and degradation of bone and cartilage are all part of the complex process of RA. This mechanism is mediated via a network of interdependent proteolytic enzymes, prostanoids, cytokines. In addition to the control these mediators generated from macrophages and other immune cells, chronic inflammatory diseases can target the alteration of arachidonic acid metabolism produced by blocking enzymes like COX , LOX.²



Fig no: 2 Formation of rheumatoid arthritis in finger bone

1.4 RA falls into one of the following categories: ¹²

- Rheumatoid spondylitis,
- Juvenile rheumatoid arthritis, and
- Palindromic rheumatoid arthritis Osteoarthritis and
- Other forms of arthritis

Juvenile Rheumatoid Arthritis:

One of several more common connective tissue disorder in the US is juvenile rheumatoid arthritis, which affects roughly 50,000 youngsters. Numerous chronic arthritis are among the main causes of functional impairment. The arthritis must start before the age of sixteen and be noticeable for at least six weeks. There is a 2:1 female predominance when both sexes are equally affected, with the exception of the systemic onset subgroup.

Despite the sole exception for the systemic onset subgroup, if both genders are equally plagued, there is a 2:1 female predominance.

Rheumatoid arthritis among kids can be classified into:

1. Oligoarticular
2. Polyarticular

Systemic Issues Several features distinguish juvenile rheumatoid arthritis from adult rheumatoid arthritis:

- The incidence for Oligoarthritis is greater.
- More frequently, systemic onset
- More frequently than small joints, large joints are affected.
- These usually have no rheumatoid nodules or rheumatoid factors.
- It is common to have antinuclear antibody Seropositivity.⁵⁶

1.5 Epidemiology of Arthritis:

Rheumatoid disorder affects 0.3% - 1% persons worldwide, with greater prevalence rates in developed nations. Women are affected three times more than men (1:3). People between the ages of 30 and 55 are typically the ones who experience it. This disorder is a chronic, excruciating condition that affects 0.5–1.0% individuals globally. Frequency of RA is roughly 0.75% in India.²⁵

1.6 Etiology⁴

Unnoticed susceptibility factors and the impact of surrounding circumstances are most likely conditions for the onset of RA. The main components are as follows:

1. The environmental factor

Smoking could contribute to the development of damaging RA. RF (Rheumatoid Factor) Present in those who are HLADRB1/SE positive. RA is linked to exposure to silicon, mineral oils, dietary factors, and blood transfusions. that is RF Present in those who are HLADRB1/SE positive, according to several studies. RA is linked to exposure to silicon, mineral oils, dietary factors, and blood transfusions.

2. The effects of sex and sex hormones

Sex hormones like estrogens, androgens & progesterone may play a part in RA susceptibility and pathogenesis because RA affects more women than men, particularly when they are younger. Pregnancy, the postpartum phase, and perimenopause are when women experience the highest occurrence.

3. **Genetic elements** Individuals having particular human leukocyte antigen (HLA) gene have higher incidence of rheumatoid arthritis than individuals not having HLA gene. However, all those possessing HLA gene do not suffer from rheumatoid arthritis.

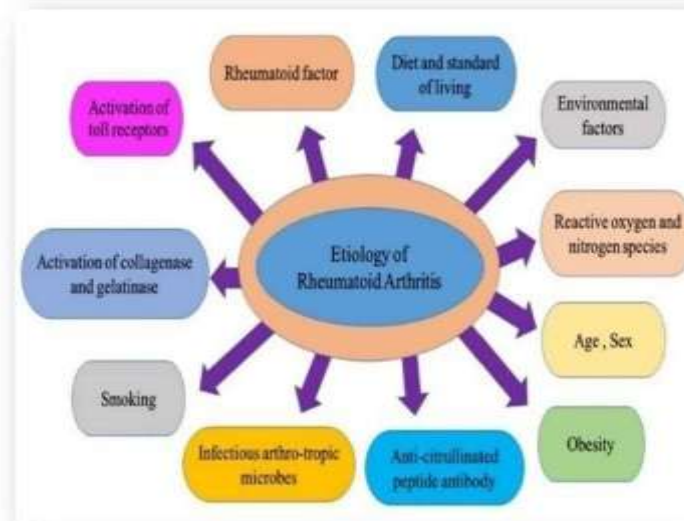


Fig no 3: Etiology of rheumatoid arthritis

1.7 The pathogenesis of Arthritis:

Pathogenesis process may go as follows: RA starts in the synovium, and the sac is formed by the membrane surrounding joint. The bag contains synovial fluid, lubricates and cushions joints in addition to nourishing and oxygenating the cartilage covering the ends of bones. Cartilage, which gives joints flexibility and stability, is composed of collagen. Rheumatoid arthritis, toxic substances caused by an aberrant immune system reaction are the cause of persistent synovial inflammation. Joint space shrinks as collagen gradually deteriorates, and eventually bone is damaged. As rheumatoid arthritis worsens, cartilage deterioration accelerates. More pannus (thickened synovial tissue) forms as fluid, immune system cells accumulate in synovium.²⁰

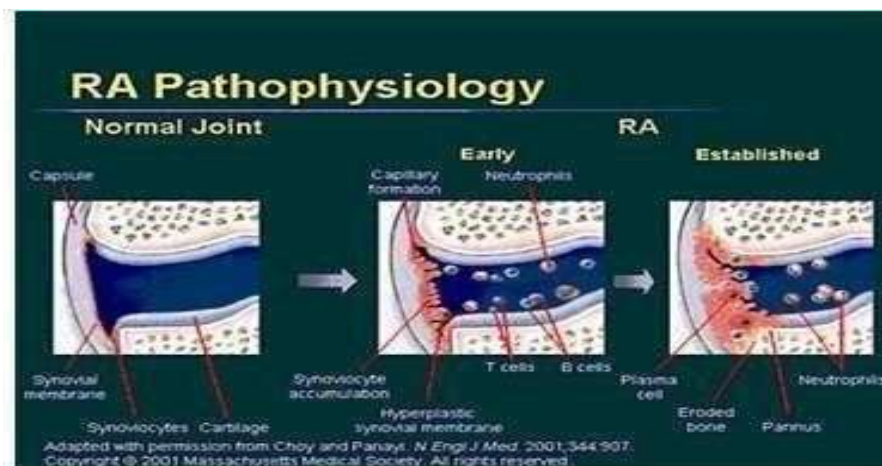


Fig no 4: Rheumatoid Arthritis Pathophysiology

PATHOGENESIS:

B cells , T cells which lymphoid cells, are two major immune system constituents that are primarily responsible for inflammation related to rheumatoid arthritis. When a T cell identifies a foreign material "non-being," releases molecules known as cytokines, which promote the development of B cells and the

generation of antibodies. These antibodies travel widely in the bloodstream, where they recognize foreign things and trigger inflammatory reactions to destroy the invader's body.²¹ The rheumatoid joint contains proinflammatory cytokines such as Interleukin-1, IL-6, IL-8, IL-15, IL-16, IL17, IL-18, IL-23, IFN- γ , TNF- α , granule Macrophage cluster activating variable, macrophages inflamed protein-1, and monocyte chemoattractant protein-1. Cytokines that suppress inflammation, include IL-4, IL-10, IL-11, and IL-13., as well as naturally occurring cytokine agonists, such as easily soluble type two interleukin-1 receptor, dissolving TNF receptor (TNF-RI), Interleukin-1 receptor inhibitor (IL-1ra), IL-18 binding protein, are responsible for maintaining the balanced activity of pro-inflammatory cytokines under normal physiological conditions. In the rheumatic joint, the equilibrium shifts in favor of proinflammatory cytokines.²² TNF- α , IL-1 stimulate endothelial cells to produce cell adhesion molecules, which can increase inflammatory cells' attraction to the active site. When IL-1 and TNF- α excite many hostile cell types discovered inside synovium, consisting Phagocytes, fibroblasts, mast cells, neutrophils, chondrocytes, dendritic cells, osteoclasts, additional proinflammatory mediators and derivative enzymes are generated. Both encourage the development of synovial cells, which causes pannus to occur. Both cytokines affect immune function by activating T and B cells.²³

1.8 SYMPTOMS:

Arthritis symptoms appear gradually. Rheumatoid arthritis may initially affect only a few joints. These are little joints found in the hands and feet. The symptoms of the condition affect more joints., Wrist, elbows, hips, knees, ankles are the Largely often Influenced locations. Rheumatoid arthritis can impact more than only the joints.²⁴

Rheumatoid arthritis can cause:

- Joints that hurt, swell, and get hot.
- Joint stiffness, which typically worsens in the morning and after a period of sleep for a minimum of forty-five minutes.
- Fatigue, appetite loss, and fever

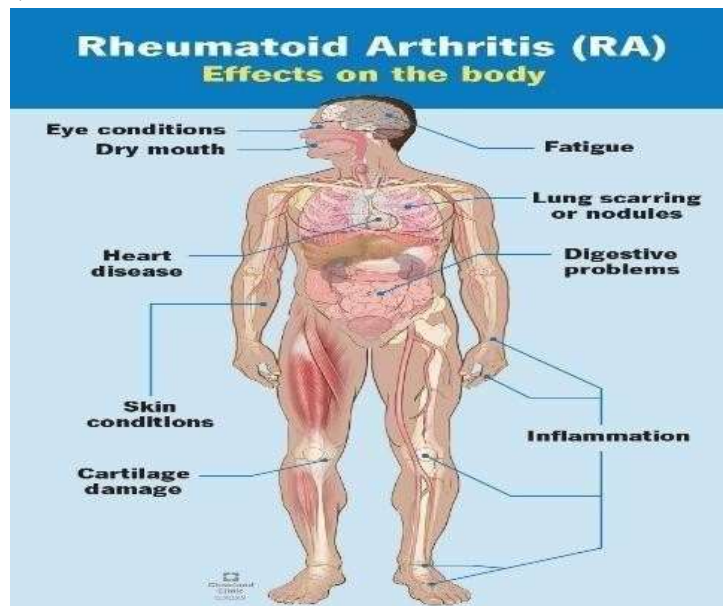


FIG No 5: Symptoms of Rheumatoid Arthritis affects the body

1.9

1.10 DIAGNOSIS:

The classification criteria for rheumatoid arthritis in the European Union is known as the Rheumatism

(EUR) classification criteria.¹⁹ The early diagnosis of rheumatoid arthritis (RA) can sometimes pose difficulties. A simple test cannot effectively diagnose rheumatoid arthritis. Both cytokines affect immune function by activating T and B cells.²³ Its early phases can be challenging. Laboratory testing and imaging techniques are commonly used for diagnosis. Determining whether the illness is mild (category 1) or severe (category 2) is crucial to ensuring that the patient receives the appropriate care even after a diagnosis.²⁵

Blood Tests: Several blood tests can be used to diagnose RA, evaluate its severity, and determine its effects.²⁵

- Rheumatoid factor.
- The test for erythrocyte sedimentation rate
- The c-reactive protein test
- Anti-cyclic citrullinated peptide antibody test.
- Testing for antinuclear antibodies.
- Tests for anaemia (low red blood cell count)
- Additional tests:
 - X-ray
 - Ultrasound
 - MR

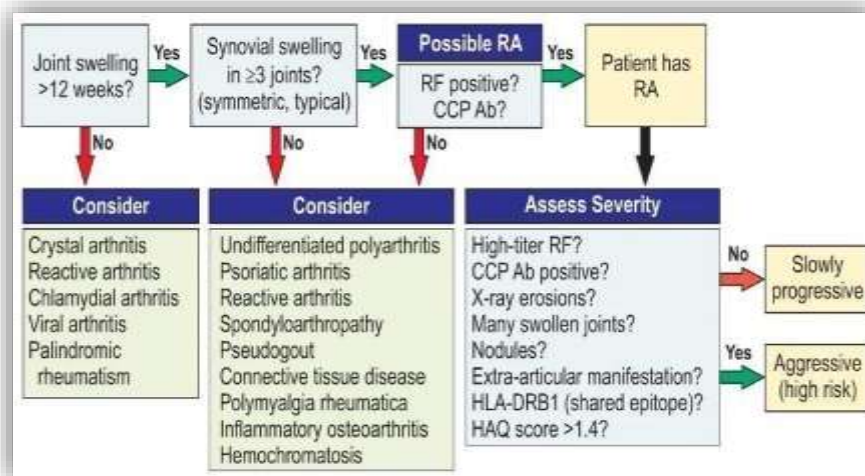


FIG NO : 6 Diagnosis of Rheumatoid Arthritis

1.11 TREATMENT:

Suppressing rheumatoid arthritis and inducing remission as soon as feasible after diagnosis, before joint degeneration begins, is the aim of treatment. Treatment plans should be based on a thorough assessment of imaging data, complications, comorbidities, and disease activity.²⁶

Following a diagnosis of rheumatoid arthritis, methotrexate, a conventional synthetic DMARD, is administered as the initial course of treatment if it is not contraindicated.²⁷

Janus kinase (JAK) inhibitors or biological DMARDs should be given if, after increasing the dosage of methotrexate to its maximum, no improvement is seen within three months or if, after six months, no remission is attained. When rheumatoid arthritis is diagnosed, methotrexate is recommended as the initial standard drug regimen if it is not contraindicated. Methotrexate mainly reduces folic acid via controlling lymphocyte and synoviocyte growth during the mitotic phase, which is when it produces antirheumatic effects.¹⁹



FIG NO: 7 Treatments of Rheumatoid Arthritis

Non-Steroidal Anti-Inflammatory Drugs:

Analgesics reduce discomfort, whereas NSAIDs reduce pain, stiffness. Drugs are often Utilized to treat the symptoms arthritis; the Validation supporting the Application of NSAIDS such as Ibuprofen, Naproxen sodium, Diclofenac, Indomethacin significantly stronger than that supporting the Application of analgesics.¹⁸ One of the most frequent side effects of long-term utilization of this medications is gastrointestinal disorders or poisoning, which usually consisting burning, belching, or discomfort, which may also lead to bleeding or the formation of stomach ulcers.²⁸

MOA of Non-Steroidal Anti-Inflammatory Drugs

NSAIDs are known to block cyclooxygenase enzymes (COX-1 and COX-2) that synthesize prostaglandins from arachidonic acid. The blocking effect results in decreased formation of prostaglandins, hence reducing inflammation and pain sensitivity. Besides their primary mechanism, NSAIDs demonstrate a secondary mechanism of action through modulation of leukocytes..

Corticosteroids:

Strong immunosuppressive and anti-inflammatory drugs are glucocorticoids for example methylprednisolone, prednisolone, dexamethasone, hydrocortisone. For the past 60 years, glucocorticoids and other corticosteroids have been used extensively to treat arthritis. Twenty Glucocorticoids reduce synovitis in the short term. Long-term glucocorticoids lessen joint damage. When taking NSAIDs and DMARDs, antiinflammatory action is required.¹⁹

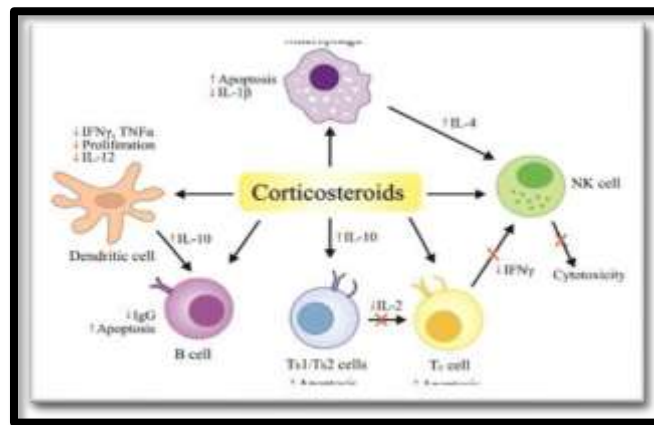


Fig no 8: Corticosteroids

MOA of Corticosteroids

Corticosteroids are able to enter the cell because of their lipid-solubility, and then act on glucocorticoid receptors (GRs) in the cytoplasm. The steroid-receptor complex enters the nucleus and binds to glucocorticoid response elements (GREs), controlling the gene expression. The corticosteroid acts on increasing the anti-inflammatory proteins, including lipocortin-1, which decreases the activity of phospholipase A2 and the formation of prostaglandins and leukotrienes. In addition, corticosteroids inhibit the inflammatory transcription factors NF-κB and AP-1, reducing the secretion of cytokines, including TNF-α, IL-1, and IL-6.

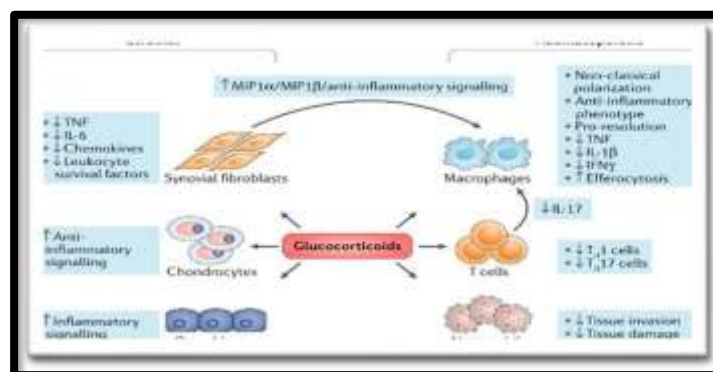


Fig no 9: Glucocorticoids

Disease Modifying Antirheumatoid Drugs:

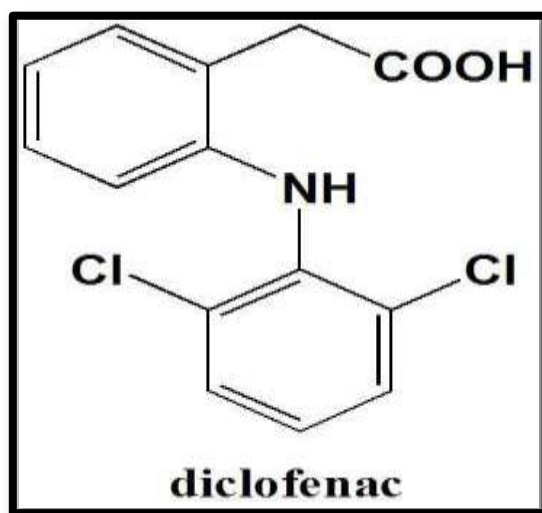
Disorder modifying anti-rheumatic medicines, or "DMARDs," are a large range of different pharmaceuticals are classified together depend on their usage, custom, and effectiveness in treating arthritis. They do not contain any particular class of medications. Its exact mode of action is unknown, however it improves function, lowers acute phase metrics, lessens joint pain and swelling, and halts the joints' ongoing deterioration. Within a year of receiving a RA diagnosis, American College of Rheumatology recommends beginning DMARD treatment.¹² Common Disorder modifying anti-rheumatic medicines, include methotrexate, sulfasalazine, and leflunomide. Methotrexate is a folic acid inhibitor that is quite affordable and relatively safe for prolonged use and has shown high efficacy. Methotrexate has no cytotoxic or immunosuppressive effects, however it does have anti-inflammatory characteristics.¹⁹

1.12 NSAIDS

Diclofenac Sodium:

One nonsteroidal anti-inflammatory drug that frequently treat inflammatory conditions such as pain, arthritis is diclofenac. It inhibits the Prostaglandins which are produced chemicals that control pain, inflammation through blocking the cyclooxygenase (COX) enzyme²⁹.

Diclofenac is present in various forms, including pills, capsules, topical gels, injections, due to its adaptability in treating a variety of medical conditions and patient preferences. It is often used for ailments like osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and excruciating pain after trauma or surgery²⁹.



Mechanism Of Action

Cyclooxygenase enzymes, specifically Cyclooxygenase-1, Cyclooxygenase-2, which create prostaglandins that induce inflammation, pain, and fever, are how diclofenac acts in arthritis. Diclofenac reduces swelling and pain in afflicted joints by lowering prostaglandin levels. By lowering the formation of leukotrienes, inflammatory cytokines, reactive oxygen species, it also affects other inflammatory pathways.³⁰

Diclofenac also has an impact on immune cells that contribute to the inflammatory response. It modifies the activity of T and B lymphocytes, reduces macrophage activation, and prevents neutrophil chemotaxis and adhesion. Diclofenac also lessens the impression of pain by desensitizing pain receptors and lowering prostaglandin levels.⁵⁷

Side Effects:

1. Diclofenac typically causes moderate side effects include headaches, nausea, rashes, and epigastric pain.
2. Diclofenac might not have an antiplatelet effect.
3. Stomach ulcers and bleeding are less frequent. Its intestinal toxicity is comparable to that of celecoxib and etoricoxib, according to certain studies.

4. Diclofenac might not have an antiplatelet effect.
5. Reversible increases in serum amino acids may lead to increased risk of heart attacks ,Strokes.
6. Renal blood flow and g.f.r. may be decreased, especially at higher dosages.
7. Diclofenac, one of the most often prescribed NSAIDs, quickly reduces pain and wound edema for a number

1.13 Animal models used to study anti-arthritic activity:

1. Complete Freund's Adjuvant induced arthritis:

Complete Freund's adjuvant (CFA) is administered to rats order for evaluate New arthritis medications. It acknowledged as the most widely used model for arthritis. The model is created through intradermal injection of a heat-killed mycobacterium solution.

The animal has T cell-mediated poly-arthritis, Heat shock proteins (HSPs) contribute to the pathophysiology of human rheumatoid arthritis by a similar manner. Both people with arthritis and those with adjuvant-induced arthritis are genetically related, rely on T cells, and have infected joints that include synovial CD4+ cells. The main cause of CFA is asymmetrical involvement of the body's major joints.³¹ Inside a rat's hind paw, there is also a noticeable rise in COX-2 levels and inflammatory mediators including TNF-1 and Interleukin-6. Elevated levels of pro-inflammatory cytokines in rat hind paw have been found to be comparable to those in arthritic patients. Another benefit of this paradigm is that the condition can be started with just one intradermal adjuvant injection. Furthermore, this model takes ten days to develop after the first administration, and the sickness peaks in severity between days 14 and 21. This is a shorter time frame than what is needed for the development of any other model of conditions, including bursitis, ankylosing spondylitis, toothache.³²

2. Formaldehyde Induced Arthritis:

An instantaneous inflammatory response occurs when formaldehyde is injected into the joint area. the migration of immune cells, such as neutrophils and macrophages, to the injection site. By acting as both an irritant and an immunogen, formaldehyde activates the immune system. This leads to the production of proinflammatory cytokines such TNF- α , IL-1 β , and IL-6, as well as the activation of numerous inflammatory pathways. Reactive oxygen species and other oxygenbased radicals (ROS) produced by formaldehyde cause tissue damage and inflammation. In this instance, oxidative stress is a significant component of the pathogenesis of arthritis. Chronic inflammation causes bone and cartilage to degenerate. Among the elevated enzymes that break down the extracellular matrix of cartilage are matrix metalloproteinases (MMPs). Bone erosion is also caused by the activation of osteoclasts, which are cells that break down bone tissue³³

1.14 PLANT PROFILE:

Cordyline fruticosa (L)



FIG NO: 10



FIG NO: 11

Introduction of Plant *Cordyline fruticosae*:

Known by several names, including Ti plant, Palm Lily, Goodluck Plant, *Cordyline fruticosa* (L.) belonging to the family Asparagaceae has Foliage that are red ,pink, orange. This plant is found throughout the world's tropical and subtropical regions and has purplish stripes and white leaf margins. This plant's roots, cortex, and leaves, for instance, are used as traditional remedies . The leaves are used to induce abortions, heal bleeding hemorrhoids, sore throats, and neck ache. In a similar vein, the roots are used to treat mammary gland infections,

laryngitis, and toothaches. Additionally, Coughing, bloody cough diarrhea, Dysentery, high temperature, urinary problems, Urine that contains blood , smallpox, insanity, and skin eruptions are all treated with this medicinal herb. Cholesterol glycosides, polyphenols, flavonoids, glucofructan, tannins, phytosterols, alkaloids, and the steroidal saponins have all been found in *Cordyline fruticosa*. According to pharmacological reports, the plant contains antioxidant, antibacterial, anti-gastric, and anti-cancer properties¹²

Cardiovascular disorders, digestive diseases, skin related disease, liver disease, rheumatoid arthritis, and This herb has also been used to cure various other conditions. which is recognized as a traditional medicinal plant. The leaves are utilized for cooking, clothes, sandals, decorations, costumes, and packing. Rhizomes can be baked into a food product that resembles molasses also consumed (Hinkle, 2007). Additionally, the herb has been used traditionally. to treat a number of illnesses: the leaves to cause abortion; the roots are utilized to treat toothaches, Laryngitis with mammary gland infections³

Cordyline fruticosa is a low-growing border shrub that is frequently utilized for decorative purposes. Oceania's peoples employ *C. fruticosa* for cultural, material, and religious purposes. There is evidence that *Cordyline fruticosa* was utilized by the early Polynesians as a food source, for religious purposes, to make costumes, and to wrap food. It was thought that surrounding the home with a hedge of *C. fruticosa* would ward against malevolent spirits it provide good fortune. In addition to its distinctive habit, shape, and exquisitely fragrant blossoms, its popularity stems from the somewhat recent development From colored leaves forms . *C. kaspar*, *C. indivisa*, *C. banksii*, and *C. pumilio* are additional species that are also referred to as cabbage trees.⁵⁴

GEOGRAPHICAL DISTRIBUTION:

Although this species is thought to have come from Papua New Guinea and Southeast Asia, early Polynesians spread it throughout most of the Pacific. In places like northeastern Australia, New Zealand, and the Pacific Islands regions it is now commonly grown and occasionally naturalized.¹⁶

TAXONOMICAL CLASSIFICATION:**Table no.1**

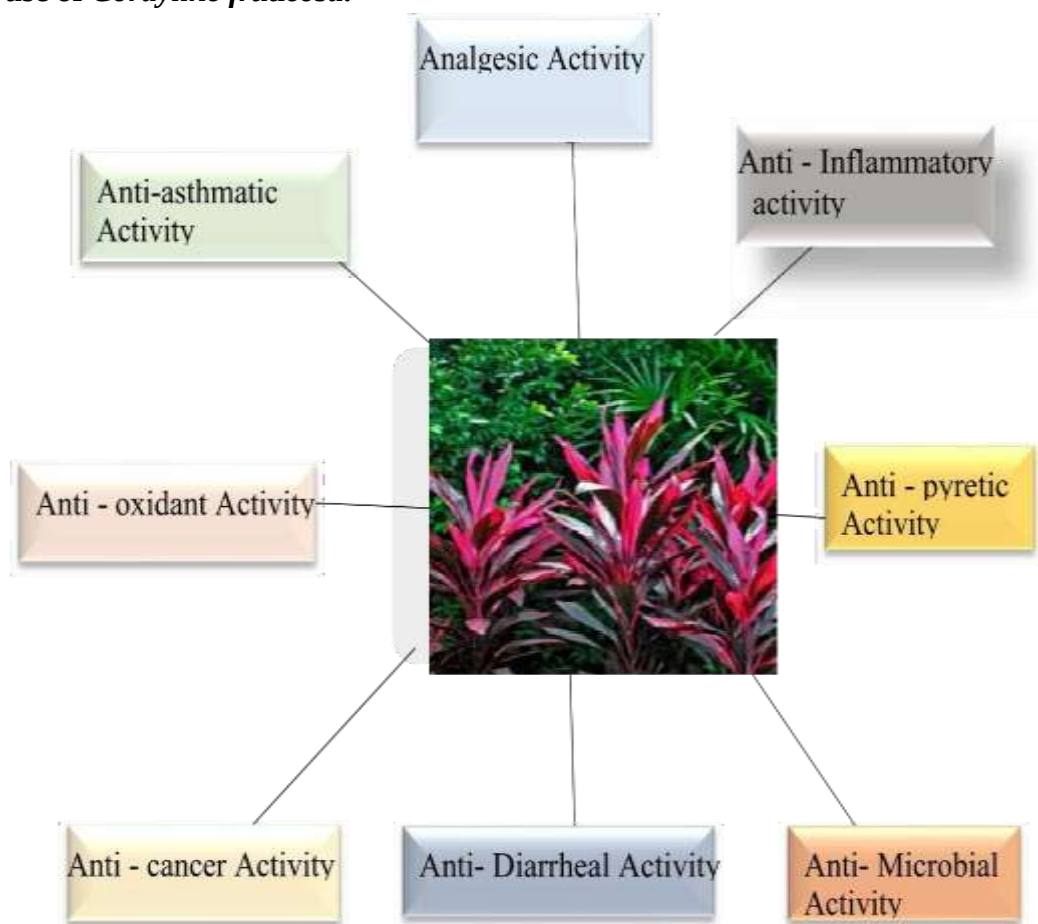
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Asparagales
Family	Asparagaceae

Genus	<i>Cordyline</i>
Species	<i>Cordyline fruticosa</i>

Conventional Applications:

Cardiac, asthmatic, neurological, and arthritic patients can benefit from the calming properties of the *Cordyline fruticosa* plant. Plant has historically been utilized as a remedy for a number of disease, concluding fever, headaches, diarrhea, coughing, hemoptysis, smallpox, insanity, and skin eruptions. Additionally, it is utilized to treat swelling pain that results from sprains, rheumatic bone pain, and joint pain. This therapeutic plant's stem and leaves were also used to induce abortions⁶.

Cordyline fruticosa flowers are used in combination with other natural medicines. Hawaiian traditional medicine that cure nose growth, asthma and shortness of breath, chest phlegm, and vomiting. *Cordyline fruticosa* leaves are wrapped around the head and chest to treat fever without sweating. *Cordyline fruticosa* leaves are utilized as therapeutic tools rather than as medications in and of themselves. They are rubbed to feverish brows, poulticed, and wrapped around warm stones to create hot packs. An infusion of heated leaves was used to induce abortion in Hawaii. In tropical West Africa, the Ti plant is used as a wound disinfectant and to treat fever, headaches, and diarrhoea. This species is utilized as a magic herb and stimulant in New Guinea to increase young warriors' ferocity¹².

Medicinal use of *Cordyline fruticosa*:**CHAPTER – 2****2. Literature review :**

Literature Survey of Anti-Arthritic Activity:**Manjusha Choudhary, et al 2014**

Manjusha Choudhary described the clinical symptoms and pathological features of rheumatoid arthritis. The symptoms of rheumatoid disorder, a systemic immune-mediated multisystem ailment, include discomfort, swelling of the synovial membrane, inflammation of the peripheral joints, morning joint rigidity, damage to joint tissues and limited joint motion. It is evident that patients have bony outgrowths that limit joint mobility as well as detrimental alterations in the cartilage and bone. Arthritis can lead to significant disability, which eventually limits a person's quality of life, makes it difficult for them to do daily duties, and results in early death.⁵ Arthritis can cause pain or inflammation in any part of the body. One of the most prevalent inflamed conditions, impacting between 0.5 to 1.0% of adults worldwide, repeated three times as many females as males affected.

Kore K.J, et.al,2011

Kore K.J discussed the currently available therapeutic approaches for the management of rheumatoid arthritis and highlighted their limitations. NSAIDs, steroidal anti-inflammatory medications, immunosuppressants, and more types are now in use. Their use is limited by possible adverse effects. The creation of novel antiarthritic medications that are safe, effective, and less toxic is currently a major concern.

P. L. Rajagopal, et.al 2019

P. L. Rajagopal explained the etiology, progression, and clinical manifestations of rheumatoid arthritis. In virtually half of the individuals, rheumatoid arthritis may start gradually and quietly. Bearing fatigue, weakness, also widespread musculoskeletal discomfort. These symptoms are probably caused by IL-1 and TNF. The joints get affected after a few weeks or months. The affected joints are painful, swollen, heated, and especially stiff when they are not moving.

Stephanie Djuichou Nguemnang, et.al.2019

Stephanie Djuichou Nguemnang explained the significance of the Complete Freund's Adjuvant (CFA)-induced arthritis model and its pathological progression in experimental rheumatoid arthritis. Research on the effects of numerous pharmaceuticals frequently uses animal models of Freund's complete adjuvant-induced polyarthritis (CFA) as rational information about rheumatoid arthritis. In fact, injecting zymosan into the rats' knee joints results in erosive synovitis with enhanced vascular permeability, neutrophil infiltration, and during the acute phase, edema and exudates develop followed by lymphocyte, macrophage infiltration, pannus formation, fibroblastic reaction typical chronic rheumatoid synovitis in the chronic phase. CFA is an immunogenic adjuvant derived from a suspension of heat-killed *Mycobacterium tuberculosis* or *Mycobacterium butyricum*. When administered at the base of the animal's tail, it causes polyarthritis to develop in a two-phase cycle of time: the first phase, which manifests as an acute local inflammatory reaction, appears in a few hours and disappears after three to five days; and the second phase, which appears after two weeks and corresponds to a chronic systemic response.

Literature Survey on *Cordyline fruticosa* :**Sharmin NAHER 1 et, al**

Sharmin Naher reported that MCFL administered at doses up to 3200 mg/kg did not produce any signs of acute oral toxicity or mortality in experimental mice. Mice treated with dosages at concentrations up to 3200 mg/kg of MCFL did not exhibit any aberrant behavior or death during the 14-day observation period. A comparable outcome was attained for the control group. This suggests that the studied doses (200, 400,

and 800) do not cause acute oral toxicity in experimental animals.

Romuald Tematio Fouedjou et al 2023

Romuald Tematio Fouedjou reviewed the traditional uses, phytochemical constituents, and pharmacological activities of *Cordyline* species. Traditional medicine has historically used cordyline species to treat a variety of illnesses, including diarrhoea, a high fever, and a bloody cough. This genus has roughly 26 recognized species names that are found all over the world. Eight species of *Cordyline* have yielded a total of 98 isolated chemicals that are classified as flavonoids, spirostane, furostane, and cholestane glycosides and may be responsible for the majority of traditional therapeutic uses. A few of these Significant antioxidant, antiproliferative, antibacterial, and hypolipidemic properties have been demonstrated by both pure substances and extracts from some *Cordyline* species. Many of these species are still a potential source of novel bioactive substances even though they have not yet been studied phytochemically or pharmacologically.

Elfita1 et al 2021

Elfita described the traditional medicinal uses, botanical characteristics, and phytochemical constituents of *Cordyline fruticosa*. One of the medicines that people use to treat infectious diseases is the decoction of *Cordyline fruticosa* stem. People know *Cordyline fruticosa* as a medicinal plant. It has been used for a time to treat many diseases. These diseases include cough bloody urine, diarrhoea, cardiovascular disease, digestive disorders, skin infections, liver cancer, arthritis and neuritis. People use *Cordyline fruticosa* to treat all these diseases because it has healing properties. The *Cordyline fruticosa* plant is very useful, for our health. The scientific name of cordyline plant is *Cordyline fruticosa* (L) A Chev, with synonyms being *Cordyline terminalis* (L) Kunth and *Cordyline fruticosa* Backer which is a plant belonging to the monoyledoneae class. It is usually grown as ornamental plants in the forest and is used to form hedges and borders for tea plantations. The compounds present in *Cordyline fruticosa* are saponins, tannins, flavonoids, polyphenols, steroids, polysaccharides, calcium oxalates, and iron.

CHAPTER – 3**AIM & OBJECTIVE**

AIM: - To evaluate the Anti-arthritic activity of *Cordyline fruticosa* in arthritic rat model.

OBJECTIVE:-

- To select, collect, authenticate, and to extract the leaves of *cordyline fruticosa*.
- To study the preliminary phytochemical investigation of the *cordyline fruticosa*.
- To determine the various active phytochemical constituents from the cordyline fruticosa by using GCMS analysis.

To study the Anti-arthritic activity by in-vitro method.

Inhibition protein denaturation assay.

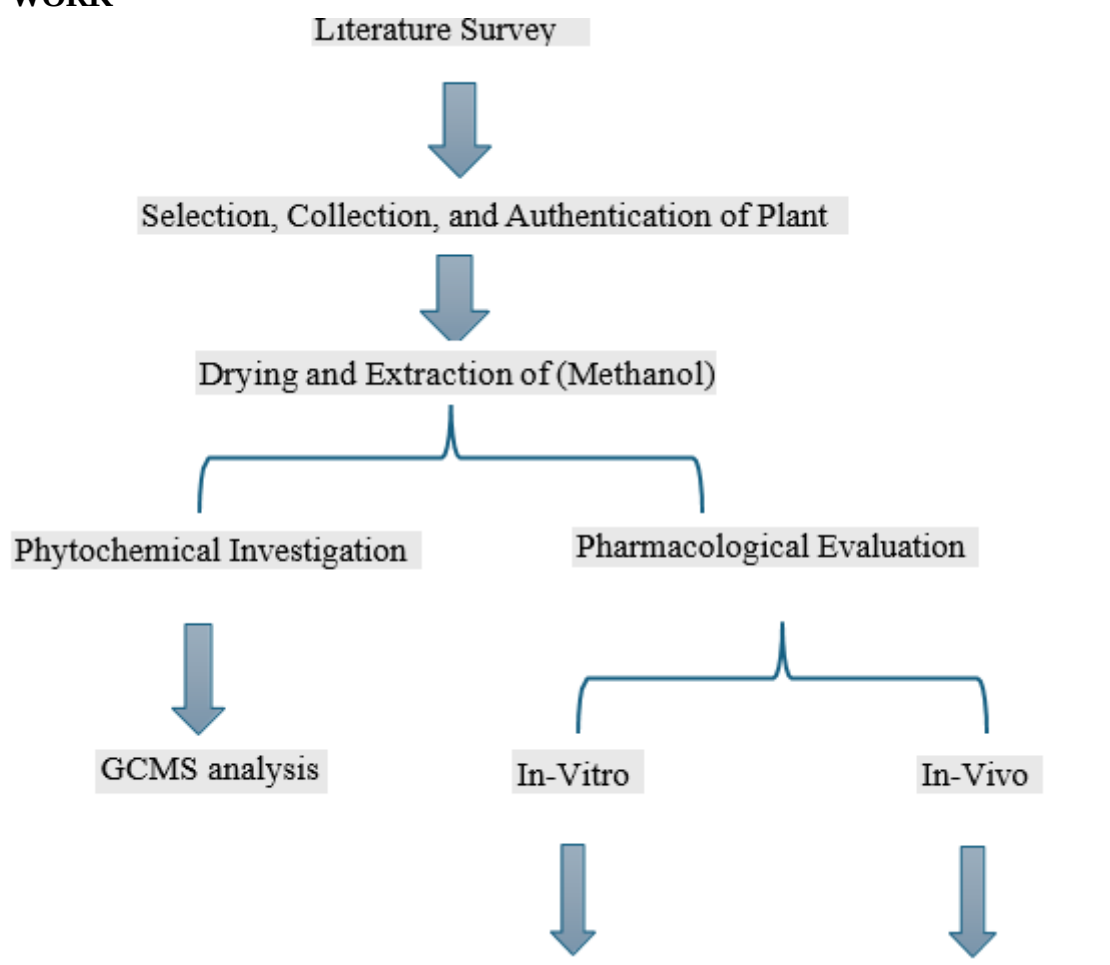
- Inhibition of egg albumin denaturation
- Inhibition of bovine serum albumin denaturation

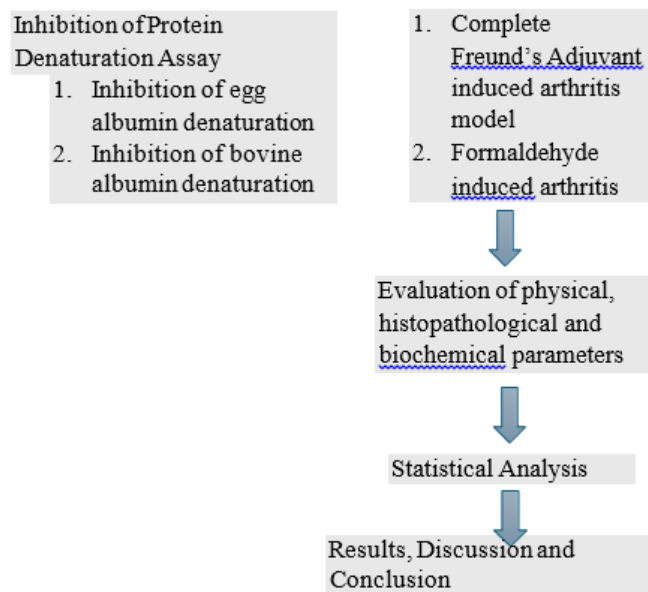
To study the anti-arthritis potential cordyline fruticosa leaves extract by using following in-vivo model.

- Complete Freund's Adjuvant induced arthritis
 - Formaldehyde induced arthritis
-

To evaluate the various parameter:

- Physical parameter: Body weight, Paw thickness, Paw volume
- Haematological Parameter: RBC Count, WBC Count, Hb Count
- Biochemical parameters: Alkaline phosphatase (ALP)/ C-reactive proteins test
- Histopathological studies

CHAPTER 4**PLAN OF WORK**



CHAPTER -5

Material & Instruments

5. Material and methods

5.1 List of utilizing Experimental Kit:

TABLE NO:2 List of utilizing Experimental Kit

Sr. No.	Kit	Mfg
1.	Alkaline phosphate reagent kit	UCCA
2.	RP reagent kit	UCCA

5.2 Drug and Chemicals Used

TABLE NO: 3 Drug and Chemicals used

Sr. No.	Drug and Chemicals	Company
1.	Methanol	Oasis laboratory
2.	Diclofenac Sodium	Sovereign Pharma Pvt. Ltd.
3.	CFA (Complete Freund's Adjuvant)	SIGMA-ALDRICH
4.	Formaldehyde	Research- Lab Fine Chem Industries
5.	1N Hydrochloric acid	Hawkins
6.	Bovine serum albumin	Probumin
7.	Normal Saline	Jedux Pharmaceuticals

8.	HCL	Research- Lab Fine Chem Industries
9.	Sodium Chloride	Research- Lab Fine Chem Industries
10.	Sodium Carbonate	Research- Lab Fine Chem Industries
11.	Alkaline Reagent Test	Research- Lab Fine Chem Industries
12.	Lead Acetate Test	Research- Lab Fine Chem Industries
13.	Ammonia Test	Research- Lab Fine Chem Industries
14.	Ferric Chloride Test	Research- Lab Fine Chem Industries

5.3 List of Instruments:

TABLE NO: 4 List of Instrument

Sr. No	Name Of Instrument	Manufacture
1.	Weighing balance	WENSAR
2.	Centrifuge	Remi
3.	Digital Vernier Caliper	ESEL
4.	Incubator	Meditech
5.	UV Spectroscopy	Simbazu
6.	Soxhlet Apparatus	J-SIL [®] 50/42

Experimental animals-

In the current investigation, Wistar albino female rats weighing 150-250 grams were used. After the period of two weeks, the animals were reused after washing periods from each group. Animal protocol was approved with approval number (SNTK/IAEC/2025/20) with the permission from IAEC of Shree Santkrupa College of Pharmacy Ghogaon (1110/PO/Re/S/07/CCSEA).

5.4 Animal used for research work:

Table No:5 Animal used for research work

Species	Wister Albino Rats
Age/Weight	9-12 Weeks (150-250 gm)
Gender	Female

CHAPTER-6

Experimental Work



6. EXPERIMENTAL WORK :

6.1 Plant collection and Authentication

The fresh plant material of *Cordyline fruticosa* were collected from a local area Turukwadi – Kokrud, Maharashtra during December 2025 Authentication of the plant were carried out by the Department of botany of Prof. Dr. N. D. Patil Mahavidyalay, Malkapur - Perid

6.2 Plant material and creating a plant extract:



FIG NO: 12 Figure of Soxhlet Extraction Process

The plant leaves were collected, washed and dried under shade. The dry material was crushed to coarse powder. About 60 g of powder weighed and placed in a Soxhlet thimble. Approximately 500-1000 mL methanol added into the round bottom flask. The Soxhlet device was built and heated to 40-65°C. The extraction continued for 4 Days until solvent became colourless. The extract filtered and concentrated

using water bath below 45°C The dried extract was weighed and stored in airtight container at 4°C for further study.

Percentage Yield:

The percentage yield for methanolic extracts of *Cordyline fruticosa* leaves (w/w) is estimated by using following formula –

$$\% \text{ Yield} = \frac{\text{Weight of obtained extract}}{\text{Weight of crude plant powder}} \times 100$$

$$= \frac{10.25}{60} \times 100$$

$$\% \text{ Yield} = 17.08\%$$

6.3 Phytochemical investigation of *Cordyline fruticosa*:

After dissolving 100 mg of methanolic extracts of *Cordyline fruticosa* leaves in five millilitres of distilled water, it was filtered. Using the following techniques, the presence or absence of phytochemicals was investigated.

TABLE NO:6 Phytochemical Investigation of *Cordyline fruticosa*

Test for Alkaloids			
Sr. No.	Test Name	Procedure	Observation
1.	Hager's test	Add 1-2 ml of Hager's reagents to a few millilitres of filtrate.	yellow colour
2.	Dragendroff's test	Add a few ml filtrate + 1-2 ml Dragendroff's reagents	A reddish brown precipitate
3.	Mayer's test	Along the test tube's sides, add a few ml of filtrate and one or two drops of Mayer's reagent.	A lightish yellow colour

Test for Glycosides			
Sr. No.	Test Name	Procedure	Observation
1.	Borntrager's test	10% ammonia solution add 3 ml of chloroform + 2 ml of filtrate	Dark yellow colour

2.	Keller-killiani test	1 ml filtrate + 1.5 ml glacial acetic acid + along a side of test tube few drops of 5% FeCl ₃ + few drops of Conc. H ₂ SO ₄	Reddish brown colour
3.	Legal test	Few drop of 10% NaOH Solution + few ml filtrate + sodium nitroprusside solution	Development of blue colour

Test for Phenolic compound

Sr. No.	Test Name	Procedure	Observation
1.	Lead acetate	Plant extract is dissolved in 5ml distilled water + 3ml of 10% lead acetate solution.	A Light yellow precipitate
2.	Gelatin test	10% NaCl, 1% gelatin solution, and 5 ml of distilled water are used to dissolve plant extract.	A white precipitate

Test for Tannins

Sr. No.	Test Name	Procedure	Observation
1.	Test for Ferric Chloride	Dissolved 50 mg of extract in 5 millilitres of distilled water and a few drops of 5% neutral ferric chloride solution	Dark green colour
2.	Bromine water test	10 ml of bromine water + 0.5 gm extract	Yellow colour

Test for Flavonoids

Sr. No.	Test Name	Procedure	Observation
1.	Aqueous sodium hydroxide test	Add a few ml of filtrate + 10% NaOH + Conc. HCl	Colourless
2.	Sulphuric acid test	Add a few ml of filtrate + Conc. Sulphuric acid	Orange colour precipitate
3.	Shinoda's test	50 mg extract, 5 ml alcohol, a few magnesium ribbon fragments, and drops of concentrated hydrochloric acid	Lightish yellow colour
4.	Lead acetate test		Yellow precipitate

5.	Alkaline reagent test	2 ml of filtrate + 2 ml of 10% ammonium hydroxide	Fluorescence yellow colour
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Test for Phytosterol and Triterpenoids

Sr. No.	Test Name	Procedure	Observation
1.	Leibermann-Burchard test	1 millilitre of filtrate with two millilitres of acetic anhydride add one or two drops of concentrated H ₂ SO ₄ (along the test tube's side)	A Deep red ring
2.	Salkowaki test	Two millilitres of filtrate and a few drops of concentrated H ₂ SO ₄	Reddish brown colour

Test for Amino acid

Sr. No.	Test Name	Procedure	Observation
1.	Ninhydrin test	2 ml of filtrate with 2 drops of a solution of ninhydrin (10 mg of ninhydrin + 200 mL of acetone)	Lightish orange
2.	Nitric acid test	Two millilitres of filtrate with a few nitric acid drops (along the test tube's walls)	Yellow colour

Test for Saponins

Sr. No.	Test Name	Procedure	Observation
1.	Foam test	Extract filtrate + 20 ml distilled water	Above 2 cm and foam height stable for 5 min.

Test for Protein

Sr. No.	Test Name	Procedure	Observation
1.	Biuret test	A solution of 2% copper sulphate with one drop, one millilitre of 95% ethanol, and KOH pellets	Lightish yellow colour
2.	Millon's test	Test 2 ml of filtrate adding a few drops of Millon's reagent	Pale yellow colour
3.	Xanthoproteic test	Filtrate + Few drops of conc. Nitric acid	Pale yellow colour

Test for Carbohydrate			
Sr. No.	Test Name	Procedure	Observation
1.	Barfoed's test	1 mL of the filtrate in addition to 1 mL of Barfoed's reagent + 2 minutes of heat	Brown sedimentation
2.	Molish's test	2 mL of filtrate with 2 drops of alcoholic α -naphthol and 1 mL of concentrated H_2SO_4 (along the test tube's walls)	Orange precipitate
3.	Seliwanoff's Test	1 mL of extract solution + 3 mL of Seliwanoff's reagent and heated on water bath for 1 minute.	Light orange colour
4.	Benedict's test	Benedict's reagent (0.5 mL) and 0.5 mL filtrate B, then boil for 2 minutes.	Dark brown colour

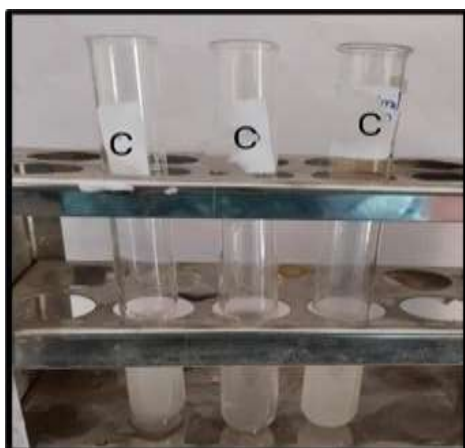
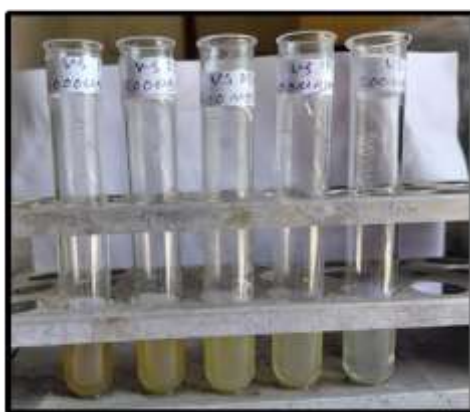
6.3 In vitro studies:

1. Egg Albumin Protein Denaturation
2. Bovine Serum Albumin Protein Denaturation

1. Egg Albumin Protein Denaturation:

The 5 mL reaction mixture included 2 mL of sample (200- 1000 $\mu\text{g/mL}$), 2.8 mL of PBS (pH 6.4) and 0.2 mL of egg albumin (from a fresh hen's egg) a comparable quantity of double-distilled water was used as control. Mixture were then heated to 70 degrees Celsius for five minutes after being incubated at 37 degrees Celsius for fifteen minutes. Using the vehicle as blank, absorbance was measured after cooling at 660 nm . The reference medications, diclofenac Sodium at 200- 1000 $\mu\text{g/mL}$, was handled similarly in order to determine absorbance.

The formula was employed to figure out percentage inhibition of protein denaturation: **% inhibition = $\frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$**

**Control****Standard diclofenac sodium*****Cordyline fruticosa* extract****FIG NO13: Egg Albumin In –Vitro Model****2. Bovine Serum Albumin Protein Denaturation Principle:**

One of the reasons for inflammation is denaturation of proteins. Potential anti-inflammatory drugs could be compounds that impede the denaturation of proteins. This test measures a compound's capacity to stop heat-induced denaturation of protein.

Materials Required:

- Test sample (extract, formulation, or compound)
- Standard anti-inflammatory drug (e.g., Diclofenac sodium, Ibuprofen)
- Protein solution: Bovine Serum Albumin (BSA) 1% w/v in phosphate buffer (pH 6.4)
- Phosphate buffer saline (PBS) pH 6.4
- Distilled water
- Test tubes
- Water bath

- Spectrophotometer (UV-Vis)
- Pipettes and micropipettes
- Incubator

Procedure:**Preparation of Solutions:****1. BSA Solution (1% w/v):** egg albumin

Dissolve 1 g of BSA in 100 mL phosphate buffer (pH 6.4).

2. Standard Solution:

Prepare a stock solution of Diclofenac sodium/Ibuprofen (e.g., 200, 400, 800, 1000 µg/mL) in distilled water or PBS.

3. Test Sample:

Prepare various concentrations (e.g., 200, 400, 800, 1000 µg/mL) in suitable solvent.

I. Experimental Setup:**1. Reaction Setup:**

- In separate test tubes, mix:
 - 0.5 mL of test sample or standard solution
 - 0.5 mL of 1% BSA solution
- For control, mix:
 - 0.5 mL phosphate buffer + 0.5 mL BSA solution
- **Incubation:** Incubate all tubes at **37°C for 15–20** minutes to allow protein-sample interaction.

3. Denaturation:

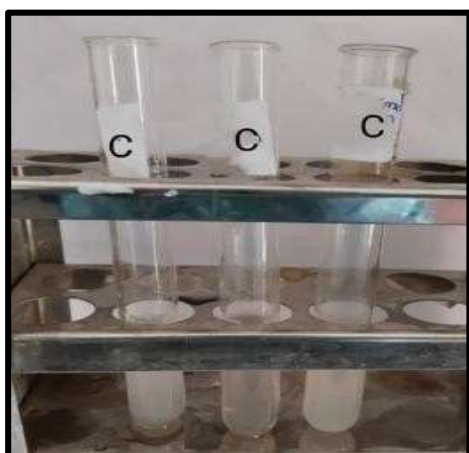
- Heat the samples at **70°C for 5–10** minutes in a water bath to induce protein denaturation.
- Cool the samples to room temperature.

4. Measurement:

- Measure the absorbance at **660 nm** using a UV-Vis spectrophotometer.

Percentage Inhibition Calculation:

Where: **Control – Test / Control × 100**



Control Shree Santkrupa College of Health and Medical Sciences, Dhule



Cordyline fruticosa extract

FIG NO: 14 Figure of In-Vitro Model of Bovine Serum Albumin

6.6 In vivo Screening methods:

For evaluation of anti- arthritis activity following In-Vivo models were used,

1. Freund's Complete adjuvant (FCA) Inducing Arthritis
2. Formaldehyde Inducing Arthritis

1 (CFA) Inducing Arthritis Induction of Arthritis:

Complete Freund's Adjuvant (CFA) Inducing Arthritis in Rats. Induction of arthritis

The complete Freund's Adjuvant (CFA) was used in arthritic rats for testing the antiarthritic potential of MCFL. In this regard, 0.1 ml of CFA was administered into the hind paws of each rat to induce arthritis. (Except of the normal control group) Four groupings of animals were divided (n = 6) and housed separate cages. Standard drug was diclofenac orally given 10mg/kg. The test substance MCFL was orally given for 28 days by dissolving 400 mg/kg dose in distilled water.

.Group I: Arthritic received 5 ml/kg for normal saline as a control;

Group II: Arthritic received 0.1 ml for CFA (Subplanter Injection) as a disease control.

Groups III: Standard treated with Diclofenac sodium 10 mg/kg after intraplantar injection of 0.1mL CFA, from the first to the 28th day.

Groups IV: Treatment with 400mg/kg MCFL after intraplantar injection of 0.1ml CFA, from the 1st to the 28th day.

TABLE NO:7 Complete Freund's Adjuvant Induced Arthritis

Sr.No	Group	Treatment	Dose	Animals
I	Control	Normal Saline	5ml/kg orally	6
II	Disease Control	Normal Saline + CFA(Complete freund's adjuvants region)	0.1ml by subplantar region	6
III	Standard	CFA + Diclofenac Sodium Orally for 28 days	10mg/kg	6
IV	Test Group (MCFL)	CFA + <i>Cordyline fruticosa</i> extract		6

		orally 28 days	400mg/kg	
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Measurement of rat paws edema:

To measure paw oedema, a digital vernier calliper was utilized on days 3, 5, 7, 14, 21 and 28 on alternate days, and a digital balance was used to record the body weight of each group. Measurement of rat paw edema in CFA-induced arthritic model; A) Normal control (normal saline 5mL/kg) B) Disease Control C) Standard (diclofenac sodium 10 mg/kg) D) MCFL 400mg/kg.



FIG NO: 15



FIG NO:16



FIG NO: 17



FIG NO:18

Haematological Profile:

Blood collection from the retro-orbital plexus was done. The blood collected was transferred into EDTA containing tubes. After blood collection, some haematological tests are performed in order to establish the levels of Hb gm/dl, RBC count, and WBC count. The serum was obtained after the centrifugation of the remaining blood samples for 20 minutes at 3000 rpm in a room temperature.

Histopathological analysis:

The hind paws were also removed for histological investigation and preserved in 10% formalin. Tissue sections were dissolved and processed with eosin and a solution of A binocular microscope was then used for the microscopic analysis. Acute and chronic inflammation, granulation tissue, and cartilage degradation were evaluated in both CFA and Formaldehyde induced arthritis

6.4.2 Formaldehyde Inducing Arthritis:

Induction of Arthritis:

Six animal groups (n = 6) were used. The vehicle was used in Group I as the normal control 5 ml/kg

normal saline . Group II was given formaldehyde as the control against disease. Group III was given the standard drug Diclofenac sodium (10 mg/kg body weight), while Group IV was given 400 mg/kg body weight of *Cordyline fruticosa*. Formaldehyde was given subplantar in the left hind paw of each animal except the normal control group. This was done to induce arthritis day one was the first day and on third day. The treatment was continued for another 28 days after the initial administration.

Group I: Arthritic animals were used as control and given normal saline 5ml/kg; Group II: Arthritic animals were used as disease control and given 0.1ml Formaldehyde (Subplanter injection)

Group III: Normal animals treated with Diclofenac sodium 10 mg/kg after intraplantar injection of 0.1mL Formalaldehyde Group IV: Administrated with MCFL 400mg/kg, Likewise, following an intraplantar 0.1 ml formaldehyde injection, starting on day 1 and day 3

TABLE NO:8 Formaldehyde Inducing Arthritis

Sr. No	Group	Treatment	Dose (Oral)	Animal s
I	Control	Normal saline	5 ml/kg	6
II	Disease Control	Normal saline + 2%v/v Formaldehyde Subplanter region	By subplanter	6
III	Standard	2%v/v Formaldehyde + Diclofenac sodium orally 28 days	10mg/kg	6
IV	Test Group (MCFL)	2%v/v Formaldehyde + <i>Cordyline fruticosa</i> leaves extract orally for 28 days	400 mg/kg	6

Measurement of rat paws edema: The paw diameter was measured at 1stday, 3rd days, 7thdays, 14thdays, 21stdays, and 28th days, respectively, using a plethysmometer and a vernier calibre. A digital balance was used to weigh the animal's body weights on the first day after induction, the fourteenth day, and the conclusion of the twenty-eighth day in order to identify the exact dosage and duration of the condition.



FIG NO: 19



FIG NO:20



FIG NO: 21



FIG NO: 22

Haematological Analysis:

Two millilitres of blood were then extracted from the retroorbital cavity on day 28. A sample of blood was taken, and haematological analyses were performed to assess the haemoglobin level (measured with Sahli's Hellige hematometer), WBC, RBC, and Hb gm/dl. The serum obtained from centrifuging the leftover blood was used at 3000 rpm and room temperature. To evaluate C-reactive protein, RA factor, and alkaline phosphatase.

Biochemical Parameter:

1. Alkaline Phosphate (ALP)

Serum alkaline phosphatase (ALP) levels were measured as a biochemical marker to assess changes in bone metabolism and joint damage associated with arthritis. The reduction/normalization of ALP levels following therapy implies that the test extract has a preventive effect against arthritic alterations.

2. C- reactive protein (CRP)

CRP or reactive protein A blood test known as C-reactive protein (CRP) is performed to determine the level of CRP in the bloodstream. The liver reacts to inflammation by releasing a substance called CRP. Inflammation, which can be caused by a variety of situations such as infections, acute illnesses, and chronic problems, is indicated by elevated blood levels of CRP.

• Analysis of Statistics

The values that are shown in in vivo research are shown as Mean $\pm$ SEM. For statistical analysis, Graphpad Prism 9.0 was used, along with a one-way ANOVA and the Dunnett Test.

CHAPTER–7

Result& Discussion



6. RESULT:**1. Preliminary Phytochemical Investigation of methanolic extract of *Cordyline fruticosa* leaves**

Tests using phytochemical analysis verify that the methanolic *Cordyline fruticosa* extract contains alkaloids, glycosides, phenolic compounds, tannins, phytosterols, teriterpenoids, flavonoids, and saponins.

Table No: 9 Preliminary Phytochemical Investigation of Ethanolic extract of *Cordyline fruticosae* leaves

Test for Alkaloids		
Sr. No.	Test Name	Result
1.	Wagner's test	+
2.	Hager's test	+
3.	Dragendroff's test	+
4.	Mayer's test	-
Test for Glycosides		
1.	Borntrager's test	-
2.	Keller-killiani test	+
3.	Legal test	+
Test for Phenolic compound		
1.	Lead acetate test	-
2.	Gelatin test	+
Test for Tannins		
1.	Ferric Chloride test	+
2.	Bromine water test	-
Test for Flavonoids		
1.	Aqueous sodium hydroxide test	+
2.	Sulphuric acid test	+
3.	Shinoda's test	-
4.	Lead acetate test	+
5.	Alkaline reagent test	+
Test for Phytosterol and Triterpenoids		
1.	Leiberman-Burchard test	+
2.	Salkowski test	+
Test for Amino acid		
1.	Ninhydrin test	+
2.	Nitric acid test	-
Test for Saponins		
1.	Forth test	+
Test for protein		
1.	Biuret test	-

2.	Millon's test	-
3.	Xanthoproteic test	-
Test for Carbohydrate		
1.	Barfoed's test	-
2.	Molish's test	-
3.	Seliwanoff's Test	-
4.	Benedict's test	-
5.	Fehling's test	-

2. GC-MS methanolic extract analysis of *Cordyline fruticosa* leaves:

GC-MS analysis of *Cordyline fruticosa* leaf extract was carried out. According to the test extract's initial phytochemical analysis, the following phytoconstituents were present:

Cordyline fruticosa leaf ethanol extract's predominant phytochemicals.

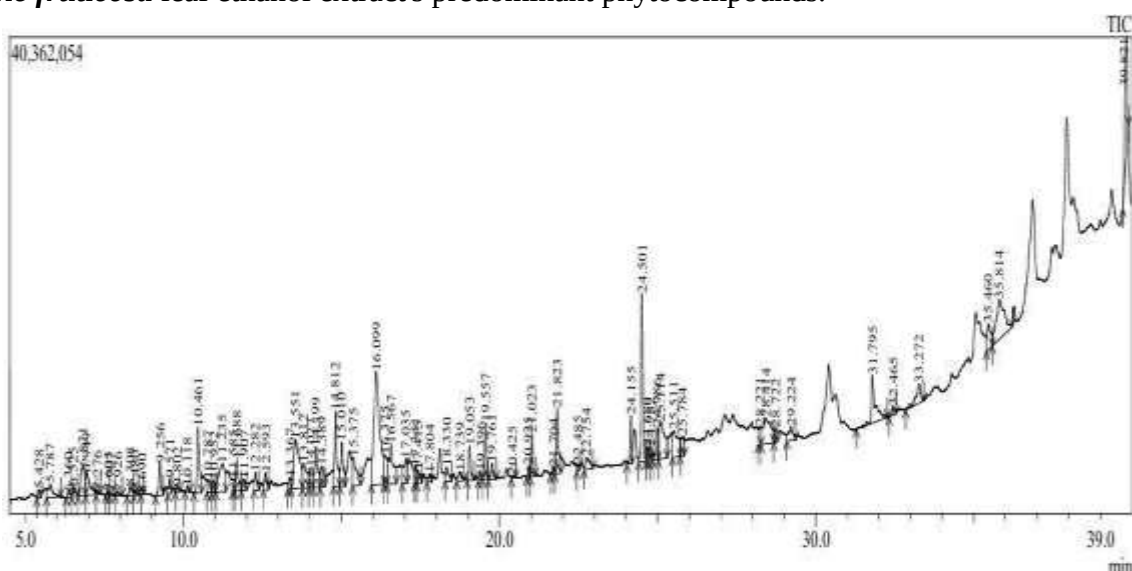


FIG NO: 23 GC-MS study of methanolic extract of *Cordyline fruticosa*.

Peak#	R.Time	I.Time	F.Time	Area	Area%	Peak Report TIC Name
1	5.428	5.360	5.495	4081052	0.35	(S)-(+)-2-Amino-3-methyl-1-butanol
2	5.787	5.675	6.120	11739130	1.00	1,2-Cyclopentanedione
3	6.360	6.285	6.470	2810292	0.24	Methyl 2,2-dimethyl-3,6,9-trioxa-2-silaundeca
4	6.525	6.470	6.670	4613772	0.39	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on
5	6.821	6.670	6.905	23409928	1.99	Glycerin
6	6.949	6.905	7.240	17654561	1.50	Oxirane, [(2-propenyloxy)methyl]-
7	7.276	7.240	7.375	1106832	0.09	D-Limonene
8	7.603	7.540	7.655	819329	0.07	3-Buten-2-ol, 1-(phenylmethoxy)-
9	7.707	7.655	7.840	871733	0.07	N,N-Dimethyl-L-leucine
10	7.926	7.840	8.070	1684820	0.14	Furaneol
11	8.308	8.215	8.435	4789267	0.41	Thymine
12	8.480	8.435	8.580	1636766	0.14	1-Penten-3-ol
13	8.690	8.660	8.730	60812	0.01	Octanoic acid, methyl ester
14	9.256	9.115	9.375	14852053	1.27	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
15	9.571	9.515	9.640	5395147	0.46	Octanoic acid
16	9.802	9.750	9.895	1893645	0.16	5-Methoxypyrrolidin-2-one
17	10.118	10.055	10.220	1722166	0.15	Pyrrolidin-1-acetic acid
18	10.461	10.335	10.580	29951269	2.55	Benzofuran, 2,3-dihydro-

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
19	10.787	10.745	10.900	8075279	0.69	2-(1-Hydroxyethyl)-2-methyl-1,3-oxathiolane
20	10.952	10.900	11.020	5831936	0.50	Carbonic acid, nonyl vinyl ester
21	11.235	11.020	11.355	33332065	2.84	Erythritol
22	11.585	11.555	11.625	3968664	0.34	1,3-Propanediol, ethyl octadecyl ether
23	11.688	11.625	11.835	16125521	1.37	4-Hydroxy-3-methylacetophenone
24	11.907	11.835	12.005	7862009	0.67	Propanoic acid, 2-[(tetrahydro-2H-pyran-2-yl)]
25	12.282	12.235	12.395	8767955	0.75	n-Decanoic acid
26	12.593	12.525	12.705	8814860	0.75	DL-Proline, 5-oxo-, methyl ester
27	13.367	13.305	13.405	4327628	0.37	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-
28	13.551	13.405	13.765	52634503	4.49	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
29	13.812	13.765	13.965	18717616	1.59	Eicosane
30	14.027	13.965	14.095	10192861	0.87	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(4
31	14.199	14.095	14.325	24142183	2.06	Undecanoic acid, 10-methyl-, methyl ester
32	14.389	14.325	14.475	11958256	1.02	2-Aminopent-4-enoic acid, N-vinyloxycarbonyl
33	14.812	14.745	14.945	32595900	2.78	Dodecanoic acid
34	15.010	14.945	15.125	21629300	1.84	2,5-Dimethoxy-4-ethylamphetamine
35	15.375	15.345	15.615	22178274	1.89	Diethyl Phthalate
36	16.099	15.945	16.345	123810281	10.55	Desulphosimigrin
37	16.375	16.345	16.445	13796050	1.18	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-
38	16.567	16.445	16.735	35060024	2.99	4,4,5,8-Tetramethylchroman-2-ol
39	17.035	16.925	17.075	14704727	1.25	Tetrapentacotane
40	17.341	17.305	17.385	5556369	0.47	Methyl tetradecanoate
41	17.415	17.385	17.545	7324160	0.62	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5
42	17.804	17.695	17.855	4584758	0.39	Tetracosane
43	18.330	18.275	18.485	8220398	0.70	Nonylamine, N,N-di(allyl)-
44	18.739	18.645	18.875	5192421	0.44	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydr
45	19.053	18.975	19.215	13052441	1.11	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-
46	19.396	19.335	19.465	3019493	0.26	Nocophytadiene
47	19.557	19.465	19.635	17489527	1.49	2-Pentadecanone, 6,10,14-trimethyl-
48	19.761	19.635	19.905	7083963	0.60	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-o
49	20.425	20.375	20.515	1030610	0.09	Triacotane, 1-bromo-
50	20.925	20.855	20.965	3432311	0.29	Tetrapentacotane
51	21.023	20.965	21.125	13856724	1.18	Hexadecanoic acid, methyl ester
52	21.704	21.615	21.745	2341928	0.20	Tetracosane
53	21.823	21.745	21.945	29633168	2.53	n-Hexadecanoic acid
54	22.485	22.445	22.525	2033853	0.17	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-
55	22.754	22.675	22.955	10018789	0.85	trans-Sinapyl alcohol
56	24.155	24.025	24.205	14698175	1.25	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
57	24.501	24.405	24.625	57217877	4.88	Phytol
58	24.680	24.625	24.735	5789291	0.49	Methyl stearate
59	24.782	24.735	24.845	4994073	0.43	Tetrapentacotane
60	24.986	24.845	25.025	14946257	1.27	9,12-Octadecadienoic acid (Z,Z)-
61	25.114	25.025	25.305	39760566	3.39	cis,cis,cis-7,10,13-Hexadecatrienal
62	25.511	25.465	25.715	26667436	2.27	Tetrapentacotane
63	25.784	25.715	25.855	12145939	1.03	Citronellol epoxide (R or S)
64	28.221	28.175	28.275	8534341	0.73	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-eth
65	28.414	28.275	28.675	38356641	3.27	Hexacotane
66	28.722	28.675	28.785	6627463	0.56	4,8,12,16-Tetramethylheptadecan-4-olide
67	29.224	29.085	29.365	11042708	0.94	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,
68	31.795	31.290	32.265	35649210	3.04	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
69	32.465	32.315	32.540	4017910	0.34	Bis(tridecyl) phthalate
70	33.272	32.840	33.415	17306423	1.47	.alpha.-Tocopherol-.beta.-D-mannoside
71	35.460	35.390	35.590	18011009	1.53	Octadecanoic acid, 2,3-dihydroxypropyl ester
72	35.814	35.590	36.215	60094469	5.12	.alpha.-Tocopheryl acetate
73	39.821	39.715	39.890	56216454	4.79	.gamma.-Sitosterol
				1173545621	100.00	

Table no – 10: GCMS Analysis

Peak No.	Compound Name	Potential Anti-Arthritic / Anti-Inflammatory Activity
1.	Phytol	Major anti-arthritic bioactive compound
2.	γ -Sitosterol	Strong anti-arthritic phytosterol
3.	4,8,12,16-Tetramethylheptadecan-4 olide	Terpenoid-like anti-inflammatory activity
4.	4-Hydroxy-3-methylacetophenone	Anti-inflammatory phenolic compound
5.	4H-Pyran-4-one derivative	Strong antioxidant activity; reduces oxidative stress
6.	5-Methoxypyrazine-2-one	Anti-inflammatory and radical scavenging activity
7.	Cyclopropanecarboxylic acid derivative	Anti-inflammatory potential
8.	2-Aminopent-4-enoic acid, N-vinyloxycarbonyl	Amino acid derivative with possible immunomodulatory role
9.	n-Hexadecanoic acid	Anti-inflammatory, lipoxxygenase inhibition
10.	2-Methoxy-2-methyl-3-(1-propenyl)-oxirane	Anti-inflammatory potential reported in epoxide derivatives

4. Impact on the denaturation of egg and bovine serum albumin:

Anti-inflammatory activity in vitro of Sample *cordyline fruticosa* was analyzed through protein denaturation technique, where the effectiveness of substances in inhibiting the denaturation of proteins by heat was examined because protein denaturation is one of the mechanisms in inflammation.

The standard drug, Diclofenac sodium, showed strong inhibitory activity with percent inhibition ranging from 72.87% to 85.27% across concentrations of 200–1000 µg/ml, confirming the reliability of the assay.

Table no - 11 : Protein Denaturation by egg albumin method

Sr. no.	Sample	Conc. µg	O. D			Mean	Percent inhibition
			I	II	III		
1	Control		0.82	0.89	0.87	0.860	
2	Standard Diclofenac sodium	200	0.25	0.24	0.21	0.233	72.87
		400	0.19	0.18	0.19	0.187	78.29
		600	0.15	0.17	0.16	0.160	81.40
		800	0.13	0.14	0.12	0.130	84.88
		1000	0.11	0.18	0.09	0.127	85.27 **
3	MCFL	200	0.67	0.65	0.66	0.66	23.26
		400	0.58	0.57	0.56	0.57	33.72
		600	0.51	0.54	0.62	0.56	35.27
		800	0.37	0.42	0.44	0.41	52.33
		1000	0.41	0.44	0.38	0.41	52.33 **

n=3

The in vitro anti-inflammatory activity of MCFL was evaluated using the egg albumin protein denaturation method, which assesses the ability of compounds to inhibit heat-induced denaturation of proteins an important mechanism involved in inflammation. The standard drug, Diclofenac sodium, exhibited strong inhibitory activity with percent inhibition ranging from 72.87% to 85.27% across concentrations of 200–1000 µg/ml, confirming the reliability of the assay. MCFL demonstrated a concentration-dependent increase in anti-inflammatory activity. At 200 µg/ml, the sample showed 23.26% inhibition, which increased to 52.33% at 800 and 1000 µg/ml. The reduction in absorbance values with increasing concentration supports its protein-stabilizing ability. However, the activity plateaued at higher concentrations (800–1000 µg/ml), indicating that maximum inhibitory effect may have been reached.

TABLE NO 12: Protein Denaturation by Bovine Serum Albumin method

Sr. no.	Sample	Conc. µg/ml	O. D			Mean	Percent inhibition
			I	II	III		
1	Control		0.82	0.89	0.87	0.860	
2	Standard	200	0.25	0.24	0.21	0.233	72.87
	Diclofenac sodium	400	0.19	0.18	0.19	0.187	78.29
		600	0.15	0.17	0.16	0.160	81.40

		800	0.13	0.14	0.12	0.130	84.88
		1000	0.11	0.18	0.09	0.127	85.27**
3	MCFL	200	0.65	0.62	0.64	0.63	79.07
		400	0.59	0.58	0.57	0.64	25.97
		600	0.52	0.53	0.51	0.58	32.56
		800	0.48	0.47	0.46	0.52	39.53
		1000	0.43	0.48	0.47	0.47	45.35**

n=3

The standard drug, Diclofenac sodium, showed strong inhibitory activity with percent inhibition ranging from 72.87% to 85.27% across concentrations of 200–1000 µg/ml, confirming the reliability of the assay. Sample–VS exhibited variable results across the tested concentrations. Although a percent inhibition of 79.07% is reported at 200 µg/ml, the corresponding mean O.D. value (0.18) appears inconsistent with the recorded absorbance values, suggesting a possible calculation or typographical error. At higher concentrations (400–1000 µg/ml), Sample–VS demonstrated a gradual increase in inhibition from 25.97% to 45.35%, indicating a dose-dependent trend. The decrease in absorbance values with increasing concentration supports its ability to inhibit protein denaturation.

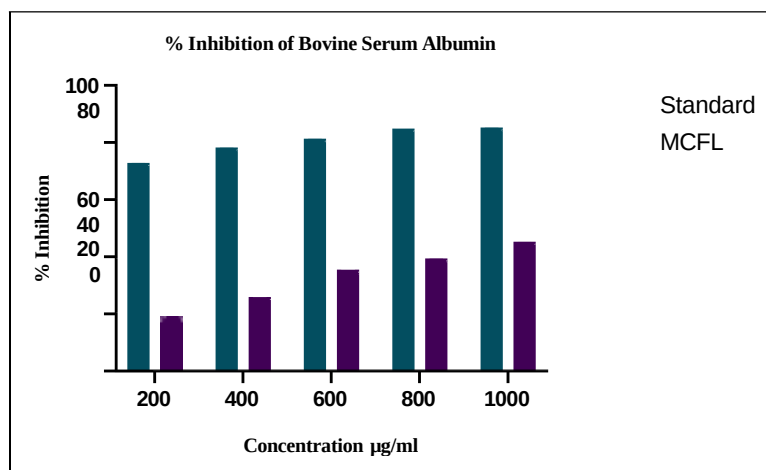


FIG NO: 26 In vitro: Protein denaturation by Bovine Serum Albumin method

5. CFA Induced Arthritis:

5.1 Physical Parameters:

5.1.1 *Cordyline fruticosa* Leaves Extract impact on the diameter of the CFA - induced arthritic paw:

In comparison with the standard control rats, the volume of Rat paw of CFA-induced arthritic control rats is much bigger. As compared with the arthritic rats, the paw edema volume in rats was remarkably decreased through the administration of 400 mg/kg of *Cordyline fruticosa* and 10 mg/kg of Diclofenac sodium.

Treatment groups	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
Normal Control	5.08±0.11	5.08±0.11	5.08±0.11	5.08±0.11	5.10±0.11	5.10±0.11
Disease Control	6.01±0.14	6.17±0.15	6.31±0.13	6.36±0.14	6.41 ± 0.13	6.46 ± 0.12
Diclofenac Sodium 10mg/kg	5.25 ± 0.20	5.30 ± 0.19	5.33 ± 0.18	5.13 ± 0.27	5.10±0.24	5.08 ± 0.22
Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	5.39 ± 0.14	5.42 ± 0.13	5.40 ± 0.14	5.40 ± 0.14	5.37 ± 0.14	5.31 ± 0.13

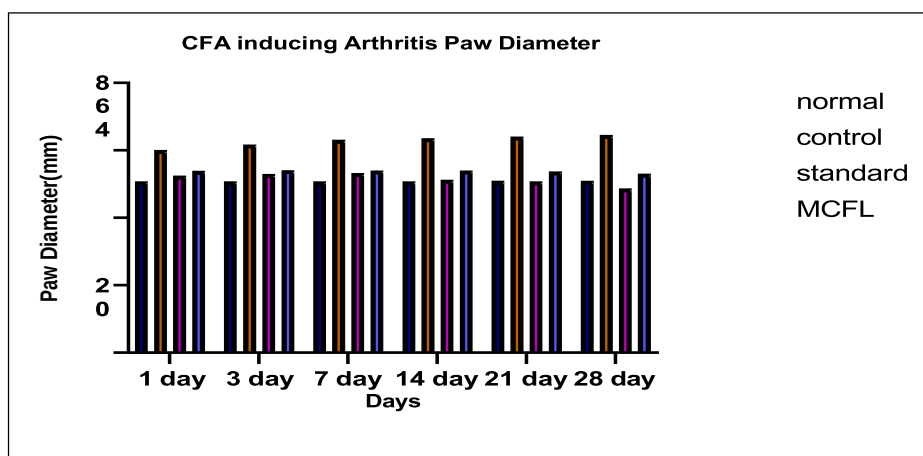


FIG NO: 29 CFA Inducing Arthritis Paw Diameter

The results (n = 6) are presented as mean ± SEM." To compare the normal control and illness control groups, one-way ANOVA was used, followed by Dunnett's test. A p-value of <0.05 was considered statistically significant. On days 14, 21 and 28, animals treated with *Cordyline fruticosa* leaf extract (400 mg/kg) and Diclofenac Sodium (10 mg/kg) had significantly smaller paw diameters (p < 0.001) than the disease control group.

5.1.2 An illustration of the Changes in body weight:

TABLE NO: 13 Impact by *Cordyline fruticosa* leaves extract on CFA induced changes in body weight:

Treatment groups	1st day	3rd day	7th day	14th day	21th day	28th day
Normal Control	194.67 ± 6.51	194.67 ± 6.51	194.67 ± 6.51	194.67 ± 6.51	194.67 ± 6.51	194.67 ± 6.51
Disease Control	203.33 ± 4.43	199.67 ± 3.90	196.17 ± 3.22	193.50 ± 2.95	191.17 ± 2.55	188.83 ± 2.48
Diclofenac Sodium 10mg/kg	194.67 ± 8.70	192.67 ± 8.61	190.50 ± 8.49	192.00 ± 8.65	192.83 ± 8.44	193.50 ± 8.43

Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	195.50 ± 6.45	192.17 ± 6.15	189.50 ± 5.99	191.00 ± 6.10	192.50 ± 6.21	193.83 ± 6.15
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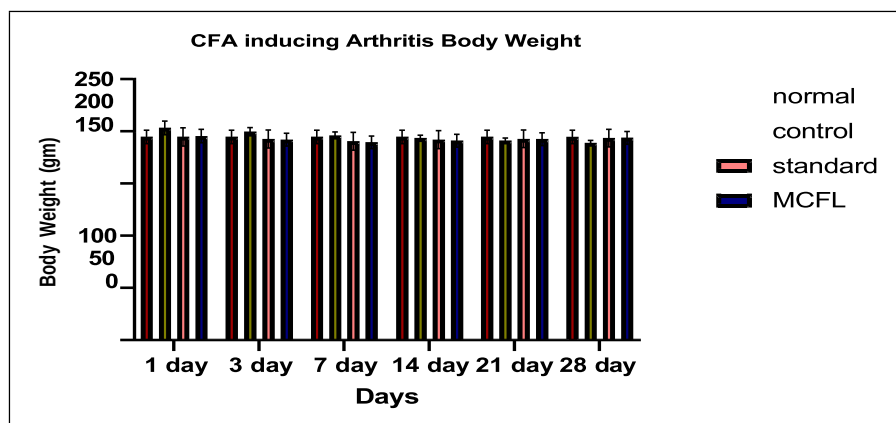


FIG No: 30 CFA Inducing Arthritis Body Weight

Body Weight Changes:

It is evident from the data collected for this study that the degree of weight reduction and the level of joint inflammation are closely related. The arthritic rats lost a significant amount of weight for the first week following adjuvant injection. In contrast, no weight loss was observed in the normal control. In the case of MCFL and standard groups, there a minor reduction in body weight from days 0 to 7; however, during days 14 to 28, there was an increase in body weight.

The data is presented as the mean $\pm$ SEM of six rats in each group. Comparing the condition-regulating group to the normal group, as well as the drug-treated group to the disease control group. A P value < 0.05 was considered extraordinary. The significance criteria are $p < 0.05$, $p < 0.01$, and $P < 0.001$, respectively. The Dunnet test and one-way ANOVA are used for comparison.

5.2 Hematological parameter:

Haematological parameter changes in arthritic rats induced by adjuvants.

On day 28 heamatological parameters are analysed . When comparing the healthy control rats with the arthritic rats, there was a significant i) reduction in the RBC count and haemoglobin and ii) an increase in the WBC count. Adjusted haematological alterations in both the developing & established phases of adjuvant induced arthritis have been significantly restored by the medication treatment.

TABLE NO: 14 Haematological Parameter: Hb count, WBC count, RBC count.

Treatment groups	Haemoglobin gm/dl	WBC X10 ³ mm ³	RBC X 10 ³ mm ³
Normal Saline	12.93 $\pm$ 0.16	10788.33 $\pm$ 252.17	6.93 $\pm$ 0.18
Disease Control	10.54 $\pm$ 0.23	12768.00 $\pm$ 175.59	5.22 $\pm$ 0.16
Diclofenac Sodium 10mg/kg	12.73 $\pm$ 0.21	12388.00 $\pm$ 313.43	6.14 $\pm$ 0.05
Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	11.37 $\pm$ 0.18	12806.00 $\pm$ 303.91	6.06 $\pm$ 0.08

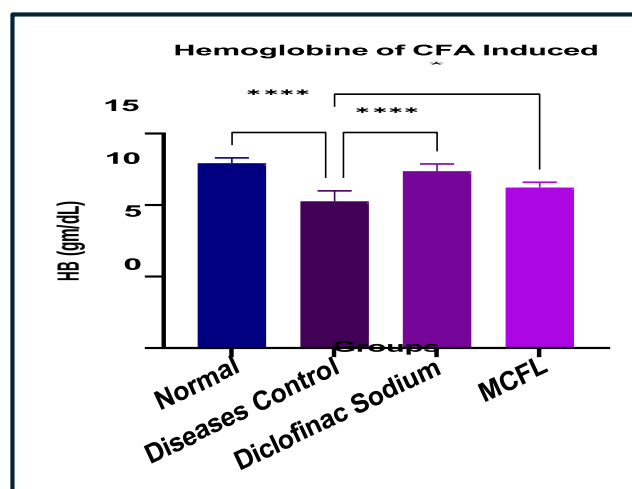


FIG No 31: CFA Inducing Arthritis hemoglobin

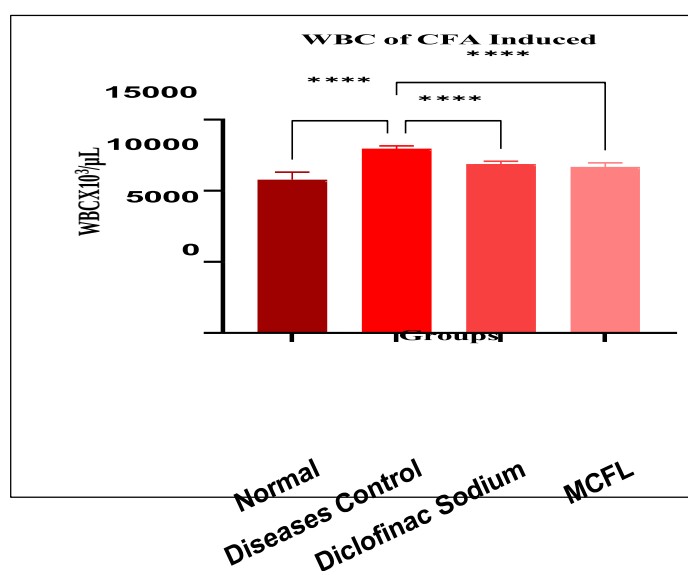


FIG NO: 32 CFA Inducing Arthritis White Blood Count

5.3 Biochemical Parameter:

Cordyline fruticosa impact on Biochemical Parameter:

Changes brought on by CFA According to the biochemical parameter results, There was a substantial ($p < 0.05$) difference between the illness control group and the normal group. CRP increases, with the former occurring more slowly. The ALP is higher in disease control alkaline phosphate than in treated alkaline phosphate ($P < 0.001$).

TABLE NO: 15 : CFA Induced Biochemical Parameter: C-Reactive Protein test, Alkaline Phosphate test

Treatment group	C-reactive Protein test (CRP)	Alkaline phosphate test (ALK)
Normal Control	4.18 ± 0.12	159.83 ± 3.28

Disease Control	7.15 ± 0.13	314.33 ± 8.39
Standard Diclofenac Sodium 10 mg/kg	5.22 ± 0.11	177.00 ± 2.44
Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	6.08 ± 0.09	200.67 ± 3.07

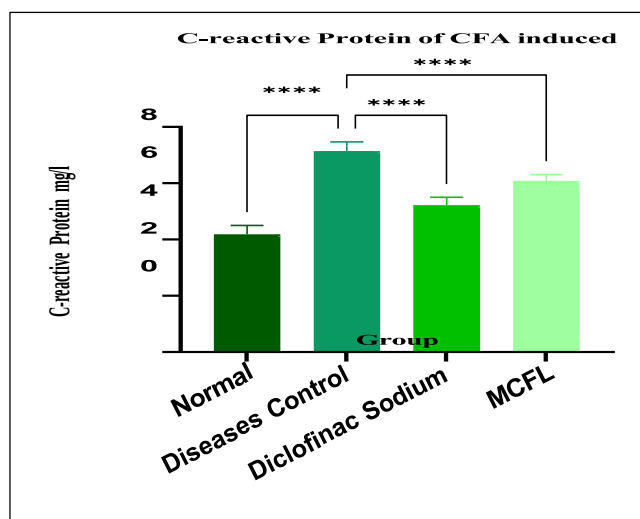


FIG No 34: CFA Inducing Arthritis C-reactive Protein test

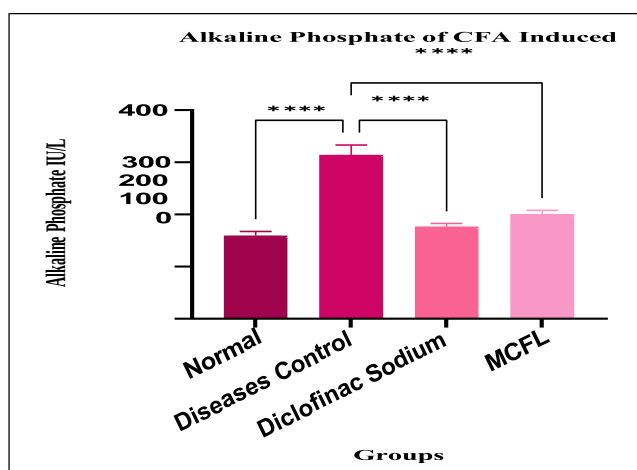


FIG No 35: CFA Inducing Arthritis Alkaline Phosphate test

5.4 Radiological analysis:

Radiological analyses were conducted on all groups, and the results showed that the standard groups had less joint swelling and no visible signs of joint deformation, whereas the arthritic control group saw considerable joint space decrease (inter tarsal joints), cartilage degradation, Bone erosion and joint deformity. The 28-day MCFL 400 mg per kg treatment resulted in less soft-tissue constriction and swelling of joint gaps than the negative control, indicating a considerable prophylaxis against bone loss. the negative control, indicating a considerable prophylaxis against bone loss.



FIG NO :36 A



FIG NO :36 B

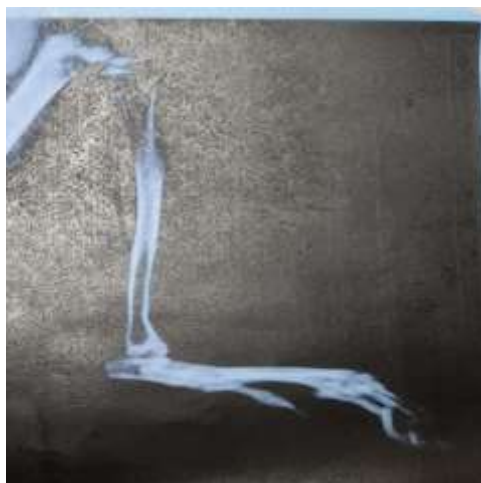


FIG NO :36 C

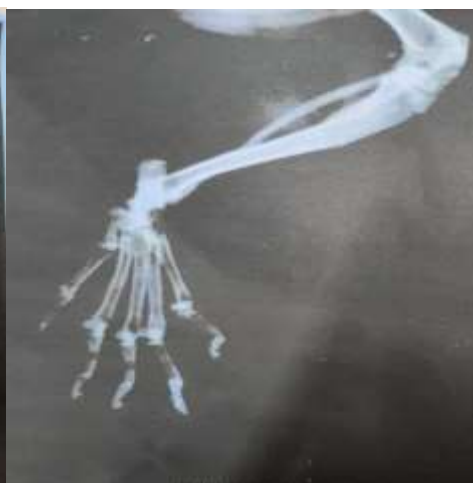


FIG NO :36 D

5.5 Histopathological Analysis:

Ankle joint histopathology results obtained from the normal control group showed that their joints were structurally normal without any cartilage degradation and without evidence of the infection or any other abnormalities. On the other hand, diseased control mice showed some evidence of pannus formation, synovial hyperplasia, cartilage destruction, and joint space injury. Synovial hyperplasia significantly improved in the group receiving diclofenac and MCFL treatment. According to the investigation's findings, MCFL can lessen paw edema joint histopathology.

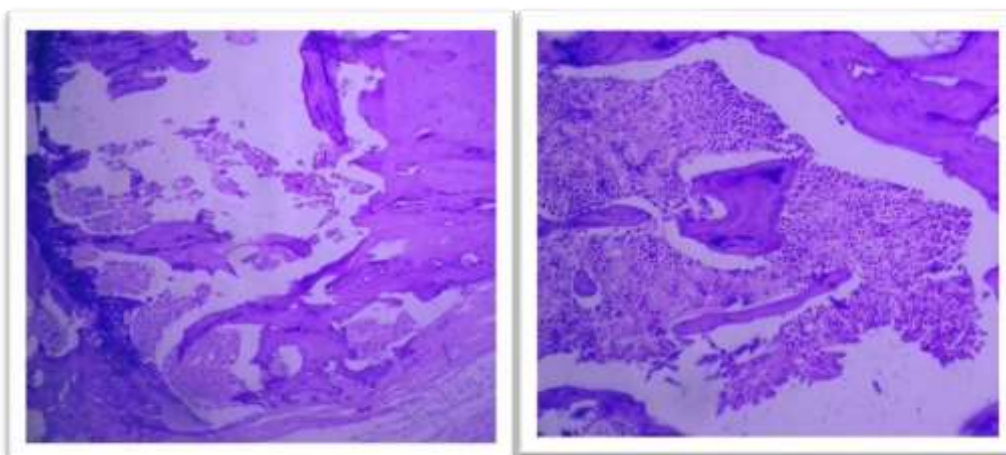


Fig no. 37A

Fig no. 37B

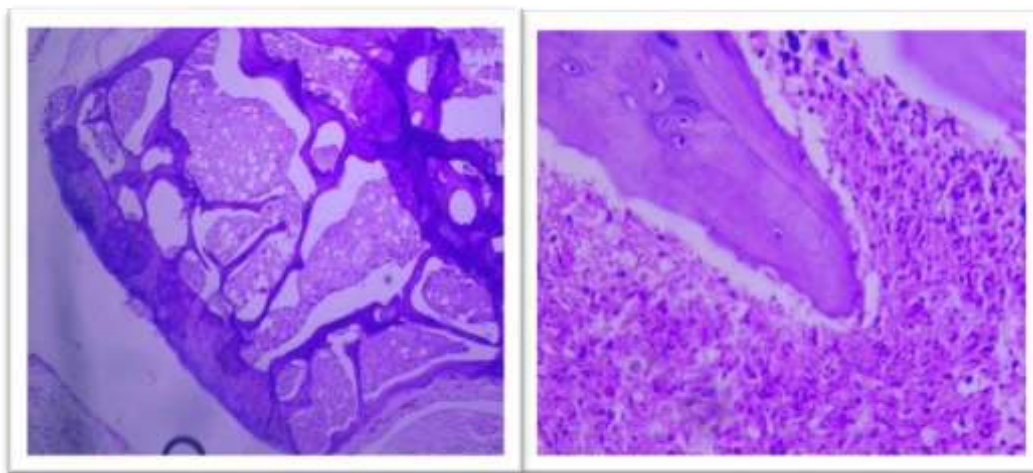


Fig no. 37C

Fig no. 37 D

(A) Normal control (B) Disease control (C) Diclofenac (10 mg/kg) & (D) MCFL (400 mg/kg) are the possible outcomes.

Pathophysiology of the joint: A) normal control B) disease control C) 10 mg/kg of Diclofenac Sodium D) 400 mg/kg of MCFL .

6. Formaldehyde Induced Arthritis:

6.1. The physical parameter:

6.1. 1. Formaldehyde-induced paw diameter is affected by *Cordyline fruticosa*

Leaves Extract:

During the monitoring period, the paw edema of all the animals increased. Rats treated with CFA-induced paw edema demonstrated a substantial ($p < 0.001$) progressive rise in paw edema in the disease control group compared to the normal group because the same paw got formaldehyde for the third time. On day 14, diclofenac sodium (10 mg/kg) generated a significant ($P < 0.001$) reduction in paw diameter. When paws treated with MCFL (400 mg/kg) and 10 mg/kg were compared to the CFA control group, paw edema decreased dramatically, particularly on days 21 and 28.

TABLE NO: 16 Formaldehyde-induced paw diameter is affected by *Cordyline fruticosa* Leaves Extract:

Treatment groups	1 st Day	3 rd Day	7 th Day	14 th Day	21 st Day	28 th Day
Normal Control	5.01 ± 0.07	5.01 ± 0.07	5.01 ± 0.07	5.01 ± 0.07	5.03 ± 0.08	5.03 ± 0.08
Disease Control	5.95 ± 0.17	5.97 ± 0.18	6.01 ± 0.18	6.05 ± 0.17	6.13 ± 0.17	6.22 ± 0.18
Diclofenac Sodium 10mg/kg	5.64 ± 0.13	5.59 ± 0.22	5.61 ± 0.22	5.57 ± 0.22	5.51 ± 0.22	5.44 ± 0.22
Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	5.84 ± 0.15	5.86 ± 0.16	5.89 ± 0.15	5.86 ± 0.16	5.82 ± 0.15	5.78 ± 0.15

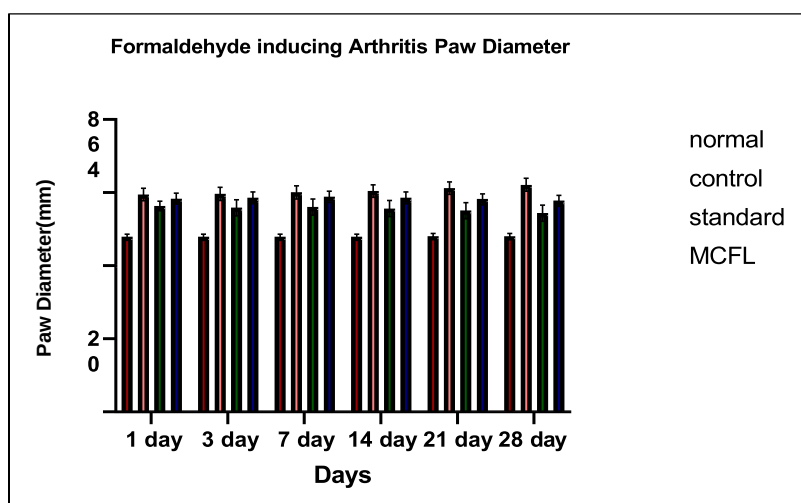


FIG NO: 36 Formaldehyde Induced arthritis Paw diameter

Mean±S.E.M is shown for each set of six rats. The normal rats are compared with the disease control rats and the diseased rats treated with drugs. The significant value for a P value is 0.05. The levels of significance are $p < 0.05$, $p < 0.01$, and $p < 0.001$. Apply the Dunnett test for one-way ANOVA

6.1.2 Changes in body weight

Throughout the following few days, there were no great differences observed in body weights of all groups of animals, whereas the disease control group witnessed a fall in body weight from days 3 to 28. In the case of MCFL and standard groups, there was a minor reduction in body weight from days 0 to 7; however, during days 14 to 28, there was an increase in body weight.

TABLE NO:17 Formaldehyde Induced Arthritis Change in body weight

Treatment groups	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
Normal Control	182.00 ± 5.86	182.00 ± 5.86	182.00 ± 5.86	182.00 ± 5.86	183.00 ± 6.08	183.00 ± 6.08
Disease Control	200.83 ± 4.85	197.00 ± 6.14	193.83 ± 5.26	195.50 ± 5.16	192.17 ± 5.20	176.67 ± 15.20
Diclofenac Sodium 10mg/kg	213.33 ± 11.50	210.67 ± 11.13	209.00 ± 10.47	210.17 ± 10.54	211.50 ± 11.10	212.00 ± 11.41
Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	177.17 ± 7.17	175.00 ± 7.13	173.00 ± 6.98	174.33 ± 7.03	175.17 ± 7.17	176.33 ± 7.12

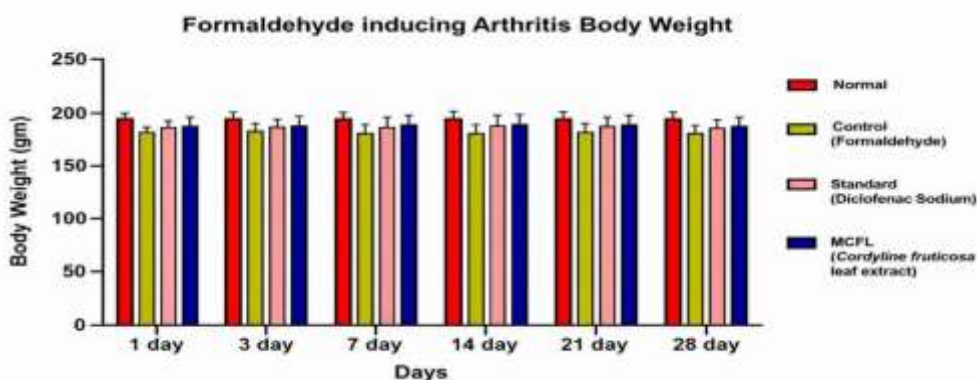


FIG NO: 37 Formaldehyde Induced arthritis Body Weight

Mean $\pm$ standard error values from each group of 6 rats are shown, compared between those who were under medication and disease controls and those who were normally kept. The results are compared using a two-way ANOVA by use of a Dunnet test with a P-value significance of 0.05. Significant levels are indicated at $P < 0.05$, $p < 0.01$ and $P < 0.001$.

6.2 Hematological evaluation:

Arthritic rats induced by formaldehyde, hematopathological values showed variation. There was a statistically significant reduction in WBCs ($P < 0.01$) and RBCs ($P < 0.001$) in rats exposed to MCFL at dose 400 mg/kg compared with the diseased control rats,

TABLE NO:18 Formaldehyde Induced Arthritis Hematological Parameter: Hb count, WBC count, RBC count

Treatment groups	Haemoglobin gm/dl	WBC X 10^3 mm^3	RBC X 10^6 mm^3
Normal Control	14.00 $\pm$ 0.26	12386.83 $\pm$ 243.49	6.92 $\pm$ 0.24
Disease Control	11.34 $\pm$ 0.24	13586.33 $\pm$ 97.84	

			4.73 ± 0.12
Diclofenac Sodium 10mg/kg	13.27 ± 0.15	13221.67 ± 30.63	5.75 ± 0.07
Methanolic extract <i>Cordyline fruticosa</i> 400 mg/kg	12.67 ± 0.10	13429.67 ± 45.86	5.45 ± 0.07

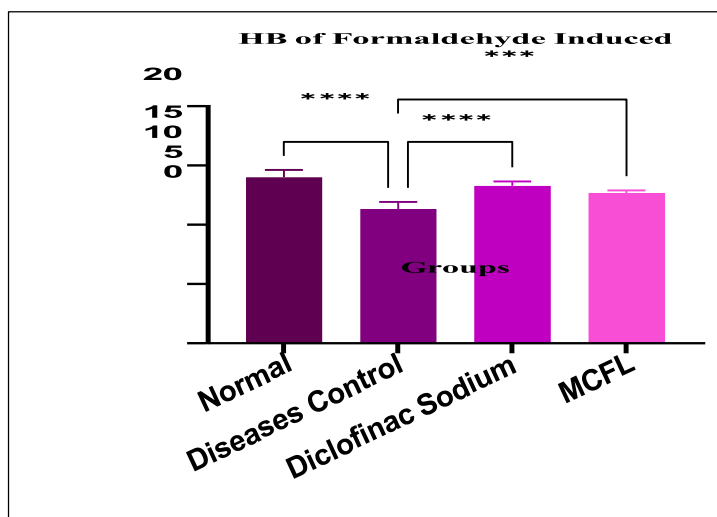


FIG NO: 38 Formaldehyde Induced arthritis Haemoglobin

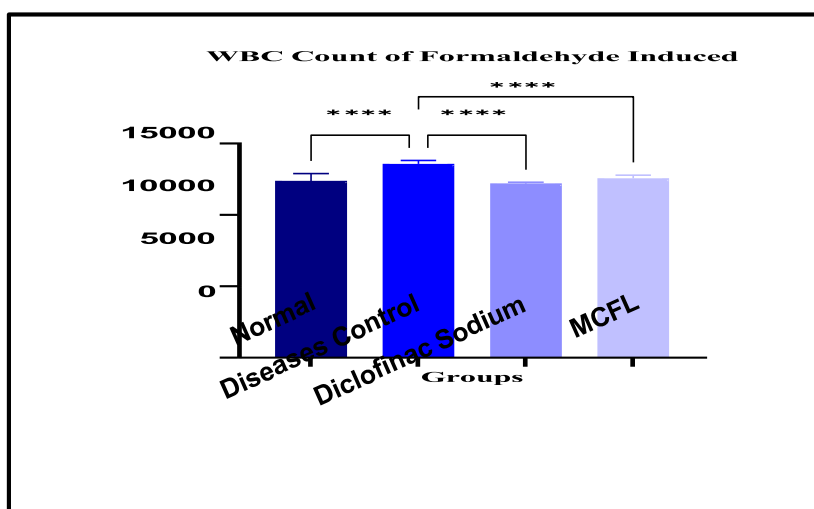


FIG No 39: Formaldehyde Induced arthritis WBC Blood Count

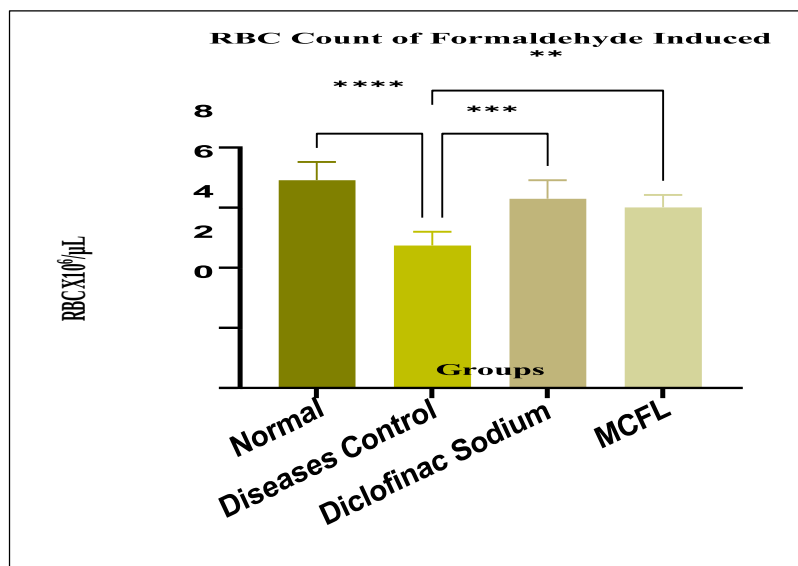


FIG No 40: Formaldehyde Induced arthritis RBC Blood Count

Mean $\pm$ SEM has been used for comparing the treated group with disease control group and normal group where $n = 6$. Where P value < 0.05 is considered significant, which is true since $p < 0.05$, $p < 0.001$, and $p < 0.001$ have fulfilled the requirements. Comparison has been made by applying one way ANOVA and Dunnet test.

6.3. Biochemical Parameters:

Effect of *cordyline fruticosa* on CFA- Induced Changes in biochemical Parameter:

The results of biochemical parameters revealed significant difference ($P < 0.001$) between normal group and disease control group, with the CRP of the latter developing more slowly.

TABLE NO: 19 Effect of *Cordyline fruticosae* on CFA-Induced Changes in biochemical Parameter: C-Reactive Protein test, Alkaline Phosphate test

Treatment group	C – reactive Protein test (CRP)	Alkaline phosphate (ALP)
Normal Control	5.42 $\pm$ 0.07	223.80 $\pm$ 8.09
Disease Control	7.30 $\pm$ 0.16	308.33 $\pm$ 11.37
Standard	5.39 $\pm$ 0.09	187.17 $\pm$ 2.04
Methanolic extract <i>Cordyline fruticosa</i> 400mg/kg	5.77 $\pm$ 0.06	195.50 $\pm$ 1.26

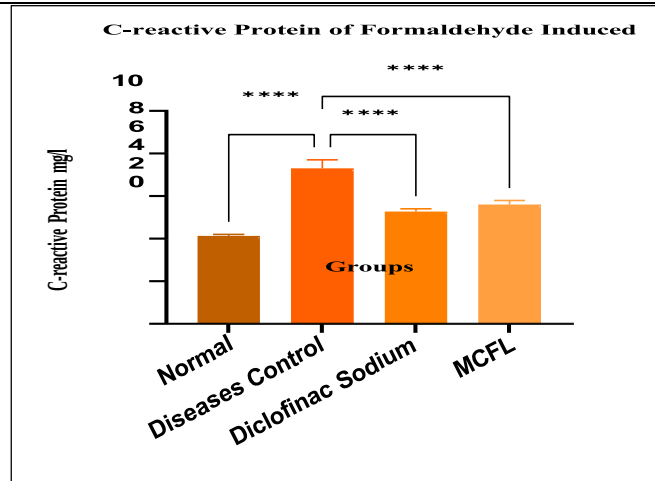


FIG No 41: Formaldehyde Induced arthritis C-Reactive Protein Test

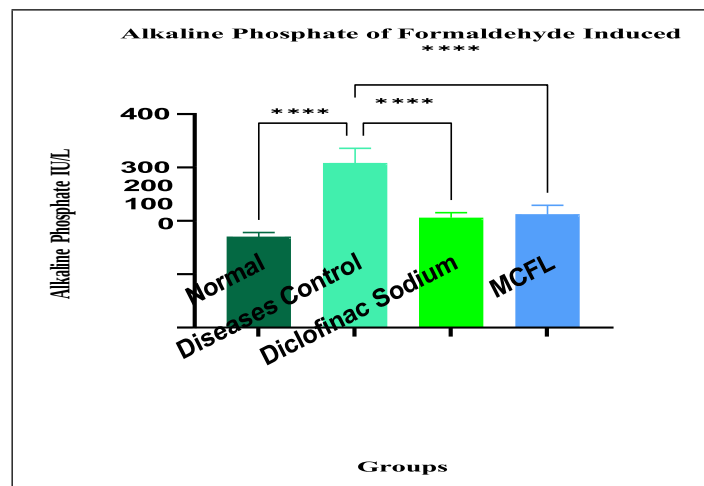


FIG NO: 42 Formaldehyde Induced arthritis Alkaline Phosphate test

Furthermore, there was not able differences between the extract group and disease control. ($P < 0.05$) Alkaline phosphate (ALP) levels in disease control are higher than in treatment ($P < 0.001$).

6.4. Radiological analysis :

The radiological assessment was done to all groups, and from the findings, it is clear that the standard groups had minimal swelling and no evidence of joint deformations. However, in the arthritic control group, there were evident reductions in joint spaces (inter tarsal joints), cartilage breakdown, bone erosion, formity There was less soft tissue constriction and swelling of joint gaps during the 28 days of treatment with MCFL at 400 mg/kg compared to the negative



FIG NO- 43 A



FIG NO - 43 B

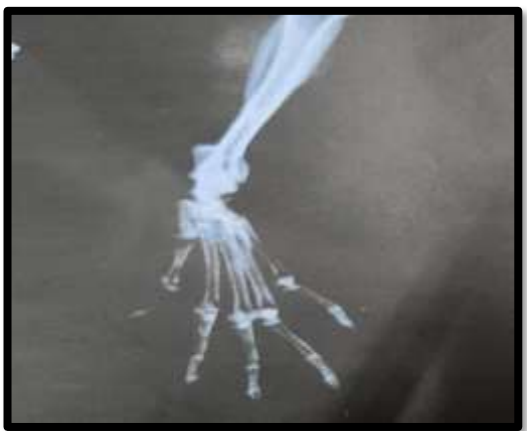


FIG NO – 43 C



FIG NO - 43 D

6.5.Histopathological analysis: ⁴³

The histological examinations of the ankle joint reports, which show significant bone degradation in the disease control group in addition to neutrophil infiltration, inflammation, and the emergence of pannus joint space damage. The treatment groups that received 400 mg/kg of *Cordyline fruticosa* and 10 mg/kg of Diclofenac Sodium exhibited joint inflammation accompanied by decreased neutrophil infiltration, as well as a decrease in pannus development and bone resorption

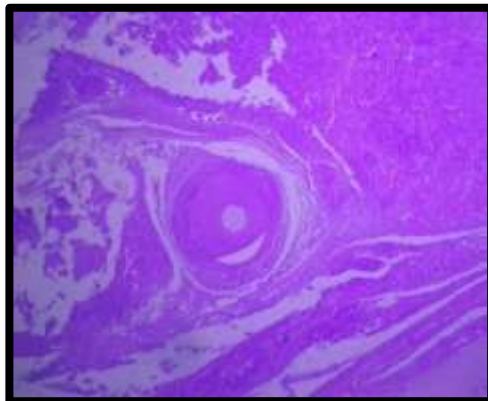


FIG NO - 44A

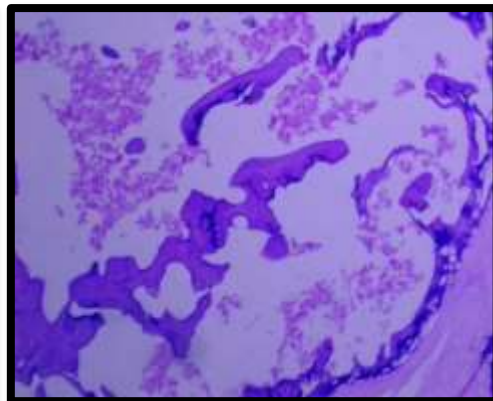


FIG NO - 44B

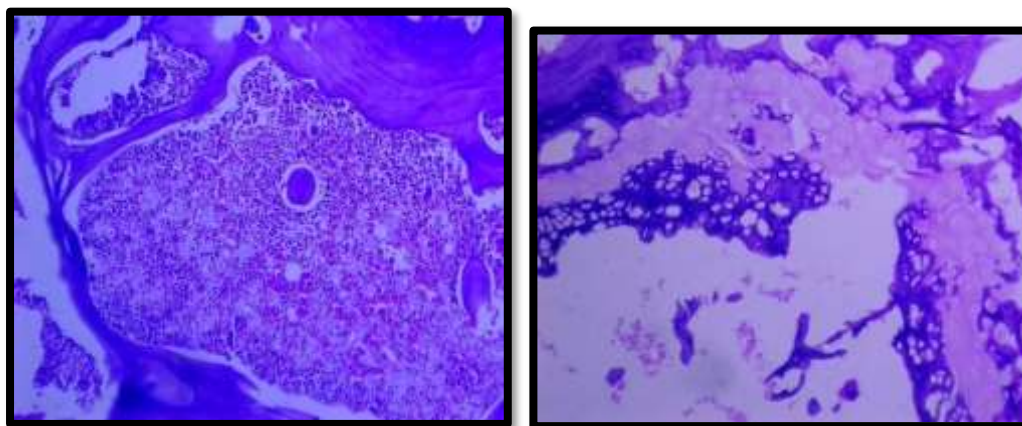


FIG NO – 44C FIG NO – 44D

DISCUSSION:

Cordyline fruticosa showed significant anti-inflammatory activity using in -vivo and in-vitro studies. The methanolic extract of *Cordyline fruticosa* underwent preliminary phytochemical screening, which demonstrated the presence of a variety of bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, saponins, steroids, and glycosides. From the literature survey it was found that these phytochemicals have anti-inflammatory, antioxidant, and other pharmacological activities.

The GC-MS study of *Cordyline fruticosa* extract identified a number of bioactive chemicals with proven anti-arthritis activity:

- D-Limonene (Peak 7) reduces inflammatory cytokines including TNF- α and IL- 1 β . It may help lessen joint inflammation caused by arthritis.
- Alpha-Tocopheryl Acetate (Peak 72): A strong antioxidant that reduces oxidative stress and inflammation, both of which contribute to arthritis progression
- γ -Sitosterol (Gamma-Sitosterol) (peak73) is one of the most powerful antiarthritic phytosterols. Inhibitors of inflammatory mediators have been shown to reduce joint inflammation and protect cartilage tissues.
- Pentadecanone (peak 59): Some ketone derivatives have anti-inflammatory capabilities, which may add to the extract's overall anti-arthritis potential.

The current study concludes that *Cordyline fruticosa* has moderate antiinflammatory activity in vitro, as measured by the egg albumin denaturation method and Bovine Serum Albumin denaturation method. The sample demonstrated a concentration-dependent rise in inhibition, with a maximum of 52.33% at higher concentrations, much lower than Diclofenac sodium. *Cordyline fruticosa* has moderate anti-inflammatory activity in vitro, as shown by the protein denaturation technique. The sample showed a dose dependent rise in inhibition, with a maximum of 45.35% at 1000 μ g/ml, much lower than Diclofenac sodium. These findings suggest that *Cordyline fruticosa* has significant in-vitro anti-inflammatory properties.

Overall, the data indicate that *cordyline's fruticosa* has strong anti-inflammatory activity. In this study, we investigated how the methanolic extract of *Cordyline fruticosa* treated rheumatoid arthritis. We conducted two animal studies: one in which arthritis was produced by CFA and the other by formaldehyde in rats. Both investigations found that the methanolic extract of *Cordyline fruticosa* is effective at treating arthritis.

In the CFA-induced arthritis and formaldehyde induced arthritis investigation, we discovered that rats treated with *Cordyline fruticosa* extract exhibited less edema in their paws than rats with arthritis. This is

comparable to what occurs in persons with rheumatoid arthritis.

The rats with arthritis lost more weight than the rats. The rats treated with *Cordyline fruticosa* extract initially lost weight, but after 14 days began to gain weight, which was better than the rats with arthritis. The study shows that *Cordyline fruticosa* has anti-inflammatory activities and can help with arthritis.

X-ray pictures revealed that treated animals had greater joint space and stronger bones. They also experienced more bone deterioration and growths than those who did not receive therapy. We also measured the thickness of their paws every day to gather more precise data on swelling. The treated animals' paws were less swollen than those in the control group. This confirmed that *cordyline fruticosa* possesses anti-inflammatory properties. Checking paw thickness every other day let us determine how effective the medication was in reducing swelling. The treated animals had edema and stronger bones. They had more joint space and less bone degeneration. The disease control group showed bone growths and degeneration. The x-ray images confirmed these findings. The treatment was effective, in reducing inflammation. *Cordyline fruticosa* showed anti-inflammatory properties.

Histopathological examination of treated animals revealed decreased joint injury and inflammatory cell infiltration. This demonstrates that *Cordyline fruticosa* preserves joint tissues and may slow the degenerative processes associated with arthritis. The histological analysis demonstrated that the treated joints saw less cartilage degradation, synovial hyperplasia, and pannus formation than the control group. These findings are crucial because they demonstrate that the plant extract can protect against the structural damage common to arthritis.

HAPTER

CONCLUSION

The present study demonstrated that *cordyline fruticosa* leaves extract showed significant anti-arthritic activity in both In vitro protein denaturation assay and complete Freund's adjuvant induced and formaldehyde induced arthritic models. The extract showed remarkable reduction in joint inflammation, arthritic scores, and paw edema. Further studies are required for the isolation and identification of active constituents and to evaluate their individual pharmacological potential.

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