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Research

Preclinical Evaluation of Hydro alcoholic extract of Corallocarpus epigaeus for Anti inflammatory activity

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Abstract

In this study the drug corallocarpus epigaeus was evaluated for acute and chronic inflammation and the results of phytochemical studies shows the presence of alkaloids, carbohydrates, glycosides, tannins, flavonoids, fixed oils, saponins, proteins, steroids present in hydro alcoholic extract. The acute oral toxicity studies shows the LD50 value >2000 mg/kg. In carrageenan induced rat paw edema method the significant percentage inhibition of paw edema was obtained. The results obtained from the complete Freund's Adjuvant induced arthritis model showed the significant inhibition of inflammation and there is no significant changes in biochemical and haematological parameters.

Keywords: Inflammation, Percentage inhibition.

INTRODUCTION

Inflammation

Inflammation is a process by which your body's white blood cells and the things they make protect you from infection from outside invaders, such as bacteria and viruses.(1) But in some diseases, like arthritis, your body's defense system -- your immune system -- triggers inflammation when there are no invaders to fight off. In these autoimmune diseases, your immune system acts as if regular tissues are infected or somehow unusual, causing damage.(2)

Inflammation Types

Inflammation can be either short-lived (acute) or long-lasting (chronic). Acute inflammation goes away within hours or days.(3) Chronic inflammation can last months or years, even after the first trigger is gone. Conditions linked to chronic inflammation include:

- Cancer
- Heart disease

- Diabetes
- Asthma
- Alzheimer's disease

CAUSES [4]

Physical:

- Burns
- Frostbite
- Physical injury, blunt or penetrating
- Foreign bodies, including splinters, dirt and debris
- Trauma
- Ionizing radiation

Biological [5]

- Infection by pathogens
- Immune reactions due to hypersensitivity
- Stress

Chemical [6]

- Chemical irritants
- Toxins
- Alcohol

Psychological:

- Excitement

Inflammation and Arthritis[7]

Some types of arthritis are the result of inflammation, such as:

- Rheumatoid arthritis
- Psoriatic arthritis
- Gouty arthritis

Other painful conditions of the joints and musculoskeletal system that may not be related to inflammation include osteoarthritis, fibromyalgia, muscular low back pain, and muscular neck pain.

Symptoms of Inflammation [8]

Symptoms of inflammation include:

- Redness
- A swollen joint that may be warm to the touch
- Joint pain
- Joint stiffness
- A joint that doesn't work as well as it should

Inflammation may also cause flu-like symptoms including [9]

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

What Causes Inflammation, and What Are Its Effects?

When inflammation happens, chemicals from your body's white blood cells enter your blood or tissues to protect your body from invaders. This raises the blood flow to the area of injury or infection. It can cause redness and warmth. Some of the chemicals cause fluid to leak into your tissues, resulting in swelling. This protective process may trigger nerves and cause pain. Higher numbers of white blood cells and the things they make inside your joints cause irritation, swelling of the joint lining, and loss of cartilage (cushions at the end of bones) over time.

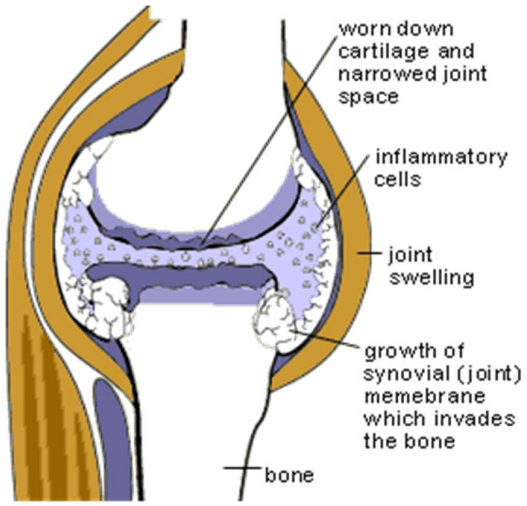

Fig 1: Inflammation in joints

Inflammation Treatment [12]

Treatment for inflammatory diseases may include medications, rest, exercise, and surgery to correct joint damage. Your treatment plan will depend on several things, including your type of disease, your age, the medications you're taking, your overall health, and how severe the symptoms are.

The goals of treatment are to:

- Correct, control, or slow down the disease process [13]
- Avoid or change activities that aggravate pain
- Ease pain through pain medications and anti-inflammatory drugs
- Keep joint movement and muscle strength through physical therapy
- Lower stress on joints by using braces, splints, or canes as needed

The acute inflammatory response [18]

Vascular changes

When tissue is first injured, the small blood vessels in the damaged area constrict momentarily, a process called vasoconstriction. Following this transient event, which is believed to be of little importance to the inflammatory response, the blood vessels dilate (vasodilation), increasing blood flow into the area. Vasodilation may last from 15 minutes to several hours. Next, the walls of the blood vessels, which normally allow only water and salts to pass through easily, become more permeable. Protein-rich fluid, called exudate, is now able to exit into the tissues. Substances in the exudate include clotting factors, which help prevent the spread of infectious agents throughout the body. Other proteins include antibodies that help destroy invading microorganisms.[19] As fluid and other substances leak out of the blood vessels, blood flow becomes more sluggish and white blood cells begin to fall out of the axial stream in the centre of the vessel to flow nearer the vessel wall. The white blood cells then adhere to the blood vessel wall, the first step in their emigration into the extravascular space of the tissue.[20]

Cellular changes

The most important feature of inflammation is the accumulation of white blood cells at the site of injury. Most of these cells are phagocytes, certain "cell-eating" leukocytes that ingest bacteria and other foreign particles and also clean up cellular debris caused by the injury. [21] The main phagocytes involved in acute inflammation are the neutrophils, a type of white blood cell that contains granules of cell-destroying enzymes and proteins. When tissue damage is slight, an adequate supply of these cells can be obtained from those already circulating in the blood. But, when damage is extensive, stores of neutrophils—some in immature form—are released from the bone marrow, where they are generated.[22] To perform their tasks, not only must neutrophils exit through the blood vessel wall but they must actively move from the blood vessel toward the area of tissue damage.[23] This movement is made possible by chemical substances that diffuse from the area of tissue damage and create a concentration gradient followed by the neutrophils.[24] The substances that create the gradient are called chemotactic factors, and the one-way migration of cells along the gradient is called chemotaxis.[25]

Large numbers of neutrophils reach the site of injury first, sometimes within an hour after injury or infection. After the neutrophils, often 24 to 28 hours after inflammation begins, there comes another group of white blood cells, the monocytes, which eventually mature into cell-eating macrophages.[26] Macrophages usually

become more prevalent at the site of injury only after days or weeks and are a cellular hallmark of chronic inflammation.

Chemical mediators of inflammation

Although injury starts the inflammatory response, chemical factors released upon this stimulation bring about the vascular and cellular changes outlined above. The chemicals originate primarily from blood plasma, white blood cells (basophils, neutrophils, monocytes, and macrophages), platelets, mast cells, endothelial cells lining the blood vessels, and damaged tissue cells.[27]

One of the best-known chemical mediators released from cells during inflammation is histamine, which triggers vasodilation and increases vascular permeability. [28]Stored in granules of circulating basophils and mast cells, histamine is released immediately when these cells are injured. Other substances involved in increasing vascular permeability are lysosomal compounds, which are released from neutrophils, and certain small proteins in the complement system, namely C3a and C5a. Many cytokines secreted by cells involved in inflammation also have vasoactive and chemotactic properties.[29]

The prostaglandins are a group of fatty acids produced by many types of cells. Some prostaglandins increase the effects of other substances that promote vascular permeability. [30] Others affect the aggregation of platelets, which is part of the clotting process. Prostaglandins are associated with the pain and fever of inflammation. Anti-inflammatory drugs, such as aspirin, are effective in part because they inhibit an enzyme involved in prostaglandin synthesis. Prostaglandins are synthesized from arachidonic acid, as are the leukotrienes, another group of chemical mediators that have vasoactive properties.[31]

The plasma contains four interrelated systems of proteins—complement, the kinins, coagulation factors, and the fibrinolytic system—that generate various mediators of inflammation.[32] Activated complement proteins serve as chemotactic factors for neutrophils, increase vascular permeability, and stimulate the release of histamine from mast cells. They also adhere to the surface of bacteria, making them easier targets for phagocytes.[33] The kinin system, which is activated by coagulation factor XII, produces substances that increase vascular permeability.[34] The most important of the kinins is bradykinin, which is responsible for much of the pain and itching experienced with inflammation. The coagulation system converts the plasma protein fibrinogen into fibrin, which is a major component of the fluid exudate. [35] The fibrinolytic system contributes to inflammation primarily through the formation of plasmin, which breaks down fibrin into products that affect vascular permeability.[36]

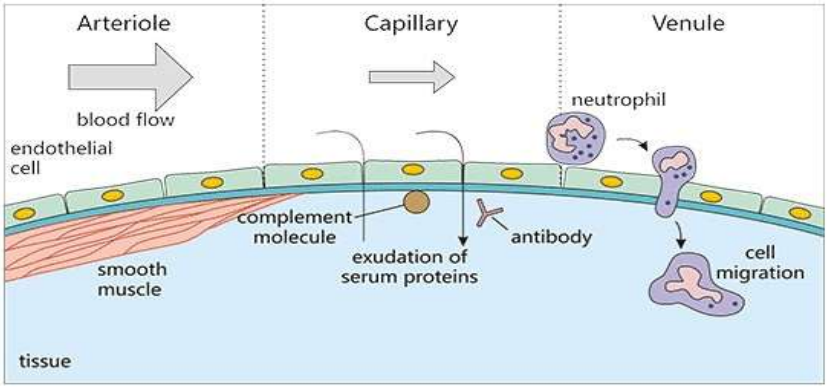

Fig 2: Fibrinolytic system generate various mediators of inflammation

Chronic inflammation

If the agent causing an inflammation cannot be eliminated, or if there is some interference with the healing process, an acute inflammatory response may progress to the chronic stage. Repeated episodes of acute inflammation also can give rise to chronic inflammation. The physical extent, duration, and effects of chronic inflammation vary with the cause of the injury and the body's ability to ameliorate the damage.[37]

In some cases, chronic inflammation is not a sequel to acute inflammation but an independent response. Some of the most common and disabling human diseases, such as tuberculosis, rheumatoid arthritis, and chronic lung disease, are characterized by this type of inflammation. Chronic inflammation can be brought about by infectious organisms that are able to resist host defenses and persist in tissues for an extended period. These organisms include Mycobacterium tuberculosis (the causative agent of tuberculosis), fungi, protozoa, and metazoal parasites. Other inflammatory agents are materials foreign to the body that cannot be removed by phagocytosis or enzymatic breakdown. These include substances that can be inhaled, such as silica dust, and materials that can gain entry to wounds, such as metal or wood splinters.[38]

In autoimmune reactions the stimulus to chronic inflammation is a normal component of the body to which the immune system has become sensitized. Autoimmune reactions give rise to chronic inflammatory diseases such as rheumatoid arthritis.[39]

The hallmark of chronic inflammation is the infiltration of the tissue site by macrophages, lymphocytes, and plasma cells (mature antibody-producing B lymphocytes. These cells are recruited from the circulation by the steady release of chemotactic factors. Macrophages are the principal cells involved in chronic inflammation and produce many effects that contribute to the progression of tissue damage and to consequent functional impairment.[40]

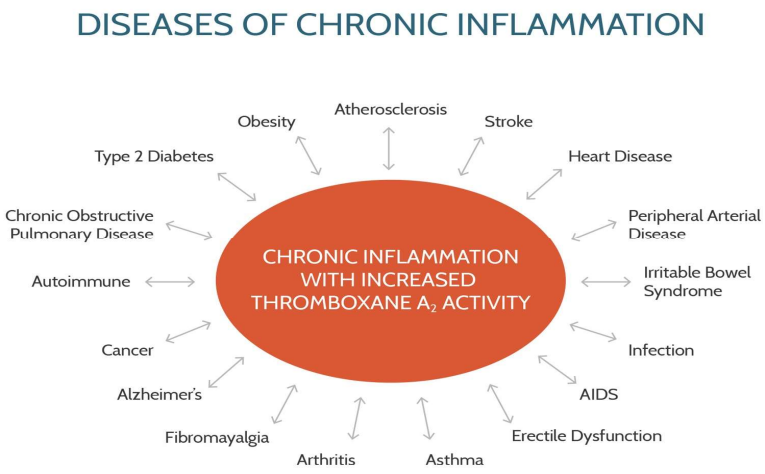

Fig 3: Disease of chronic inflammation

Plant profile

Fig 4: *Corallocarpus Epigaeus Linn*

BOTONICAL NAME: ***C****orallocarpus epigaeus*.
SYNONYMS: *Aechmandra epigaea, Bryonia epigaea, Corallocarpus gracilipes*.
COMMON NAME: Red fruit Creeper.
FAMILY: Cucurbitaceae (Pumpkin family).
SPECIES: *Corallocarpus epigaeus* (Rottler) C.B.Clarke.
TAMIL NAME: Aagasa Karudan Kizhangu.
GENUS: Corallocarpus.
Corallocarpus epigaeus (Arn.) Cl. (Cucurbitaceae) known as Jungali suran in Gujarat. It is distributed in tropical Africa, Persian Gulf region and India (Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal).
Monoecious, up to 4m long with tuberous root climber. Stem angular-sulcate and tendrils simple, elongated and glabrous. Leaves 3 lobed, 20-60mm long, cordate, finely hairy on both surfaces and lobes obovate to oblanceolate. Petiole 10-30mm long, glabrous to shortly hairy. Male peduncle 4-6cm long, 5-15-flowered, pedicels 1-5mm long, filiform. Calyx lobes lanceolate, 1mm long. Corolla greenish yellow,obtuse and 1mm long. Female flowers often solitary, on contracted axillary branch,pedicel 1-5mm long, thickened in fruit;calyx-tube campanulate, 2 mm long; petals 1.5-2.5mm long, 1-1.5mm broad, reflexed. Fruit ovoid or ellipsoid, beaked and glabrous. Seeds

asymmetrically pyriform, smooth,yellowish and turgid. Flowers and fruiting on June to October [103]. The root contains a bitter principle allied to bryonin. A phydroxybenzoyl ester, named epigaeusyl ester,a sesterpene lactone, viz. corallocarpscalaride,a pyridine carboxylic ester, designated as corallocarpeonyl ester [104]. It has a bitter and sub-acid taste and is credited with alterative and laxative properties, and is used in syphilitic rheumatism, later stages of dysentery. Deccan and Mysore the root has repute as a remedy for snakebite administered internally and applied to bitten part [105].

MATERIALS AND METHODS

Plant material

The plant was collected from Tamil Nadu forest department, Alagar kills, Madurai , during the month of September 2023. The plants were identified and authenticated from Department of Botany, American college,Madurai. A voucher specimen (212) of the plant was deposited in the department of Pharmacognosy, K.M.College of Pharmacy,Madurai. The collected plant was washed with distilled water to remove the dust and adhering materials and then was dried under shade. The shade-dried material was powdered by means of mechanical grinder and the powder was allowed to pass through sieve no. 60 for powder microscopy. The coarse powder was used for extraction.

Drying

The Rhizome of *Corallocarpus epigaeus* were washed and dried under shade for 15 days on fresh cotton cloth. After 15 days the dry weight is taken.

Grinding of the plant for extraction

Cleaned and mixer was used for grinding the plant materials. After proper grinding, the weight of the powder was obtained. These powders were used for hot extraction.

Solvents order and temperature:

Petroleum ether	:	60º-80º C
Chloroform	:	60º-62º C
Hydro alcohol	:	100º C

Preparation of extracts of Rhizome of *Corallocarpus epigaeus* by hot continuous percolation method

The flask with the given solvent is heated to a particular temperature. The vapour produced passes through the siphon tube into the thimble kept above where it is condensed and tickles down into the flask again through the thimble dissolving the active constituents in it. The method is described as the continuous extraction. The process is continued until all the soluble constituents get separated. The extract at the bottom was collected and dried under reduced temperature and pressure. Each time, before the extraction with other solvents, the powdered substance is air dried.

About 200 gm of dried powder was properly packed in Whatmann filter paper (grade no.1) and kept in thimble and the soxhlet apparatus was set up. The extraction of powder was done with different solvents with solvents of increasing polarities like petroleum ether (6080º C), chloroform, and hydro alcohol. Here temperature maintenance is based on the solvents used for extraction. The solvents were removed under reduced pressure using rotary evaporator and stored in desiccators. The consistency of the extract is semi solid. (This method is repeated until getting desired extract).

Flow chart of extraction of Rhizome of *Corallocarpus epigaeus*

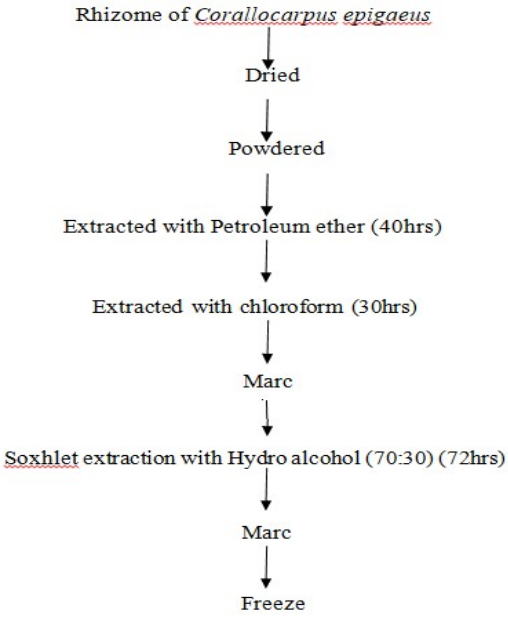

Fig 5: Flow chart of extraction of Rhizome of *Corallocarpus epigaeus*

Table 1: Percentage Yield of Various Extracts of Rhizome of *Corallocarpus epigaeus*

S. No.	Solvent used for Extraction	Time required for complete extraction (Hrs.)	Colour of extract	Percentage yield of (w/w)
1.	Petroleum ether	40	Dark green	7.20%
2.	Chloroform	40	Dark greenish brown	9.80%
3.	Hydro alcohol (70:30%)	72	Dark brown	15.30%

The crude extract of Rhizome of *Corallocarpus epigaeus* were subjected to qualitative tests for identification of various plant constituents.

Pharmacognostic studies

Proximate analysis

Determination of moisture content and total solid content

The percentage of moisture content for both raw plant materials has been calculated because any excess water in medicinal plant materials would promote microbial growth, presence of fungi or insects and cause degradation after hydrolysis. [106-109] The percentage of moisture content in a previously dried and tared flat weighing container was measured gravimetrically in which 5 g of precisely weighted air-dried material was put in. The sample was dried in an oven at 100°C-105°C until 2 consecutive weighing did not differ by more than 5 mg. Total solid content was determined by the following formula: Total solids (%) = 100 - Moisture (%)

Determination of ash values

These are used to determine a crude drug's consistency and purity, and to establish its identity. That medication contains definite amounts of inorganic radicals, such as phosphates, carbonates and sodium silicates, potassium, magnesium, calcium etc. Quantitative determination in terms of different ash values therefore helps in their standardization as well as in determining foreign inorganic matter that is present as impurity.

Determination of Total ash

Four grams of the powdered material were precisely weighed and put in a crucible of silica which was previously ignited and tared. The material was spread out in an even layer and ignited by gradually raising the

heat to a temperature of 500 - 600°C until it was white, indicating the carbon absence. The material was refrigerated and measured in a desiccator.
The total ash content in the air-dried material was calculated in mg / g.

Determination of Acid-insoluble ash

In addition, 25 ml of hydrochloric acid was applied to the crucible containing complete ash, covered with a watch glass and gently boiled for 5 min. The watch glass was rinsed with 5 ml of hot water and added the liquid to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until neutral to the filtrate. The insoluble material left on the filter paper was transferred to the original crucible, dried on a hot plate, and ignited to weight constant. The residue was allowed to cool for 30 min in a suitable desiccator, then weighed without delay. The amount of acid-insoluble ash in air-dried material was measured in mg / g.

Qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites such as alkaloids, carbohydrates, glycosides, tannins, and phenolic compounds, flavonoids, fixed oils, saponins, proteins and amino acids, and steroids present in the hydroalcoholic extracts of *Corallocarpus epigaeus* **(110)**

Alkaloids

The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicated the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was recorded if heavy precipitate or flocculation was observed **(111)**

Flavonoids Shinoda test

The presence of flavonoids was estimated by Shinoda test. The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* were treated with few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red colour within few minutes indicated the presence of flavonoids. **(112)**

Alkaline reagent test

The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.

Cardiac glycosides

Keller-kiliani test was performed for the presence of cardiac glycosides. The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was treated with 1 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few drops of concentrated H_2SO_4 were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides **(113).**

Plobotannins

The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatanins **(114)**.

Saponins

The presence of saponins was determined by Frothing test. The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5 cm indicated the presence of saponins **(115)**.

Steroids

Liebermann-Burchard reaction was performed for the presence of steroids. A chloroformic solution of the crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was treated with acetic anhydride and few drops of concentrated H_2SO_4 were added down the sides of test tube. A blue green ring indicated the presence of steroids.

Tannins

The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue color indicated the presence of tannins [116].

Triterpenes

The crude powder and hydroalcoholic extract of Rhizome of *Corallocarpus epigaeus* was treated with concentrated sulphuric acid (H_2SO_4). Appearance of reddish-brown ring indicated the presence of triterpenes.

Quantitative phytochemical analysis

Determination of total phenol content

The amount of total phenol content was determined by Folin-Ciocalteu reagent method [117]. 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) was mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate was added. The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 760 nm. The total phenolic content is expressed in terms of gallic acid equivalent (mg/g of extracted compound).

Determination of flavonoid content

The amount of total flavonoid content was determined by Aluminum chloride method [118]. The reaction mixture consisted of 1.0 ml extract, 1 ml methanol, 0.5 ml aluminum chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

Pharmacological evaluation

Animals

The study was conducted on female Wister rats (175-200gm) housed in polypropylene cages under standard conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12h light/dark cycle) were used. They were fed with standard diet and water. The food was withdrawn 18 hours before the experiment but allowed free access of water. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The experimental protocol is duly approved by the institutional ethical committee.
(IAEC/SHAHULIMTHIYAS.M/TNMGRMU/M.PHARM/261621500506/KMCP/21/2023-2024)

Acute oral toxicity studies

Acute toxicity was carried out according to Organization of Economic Co-Operation and Development (OECD) no 425 guidelines and LD50 values was estimated to be >2000mg/kg. Based on the results obtained from this study, the doses of further pharmacological studies were fixed to be 200 and 400mg/kg [119].

Anti-inflammatory activity Carrageenan induced rat paw edema[120]

Rats were divided into four groups each consisting of six Rats. The treatment regimen was as follows:
Group I (Control): Vehicle (2ml/kg, p.o), of normal saline.
Group II (Standard): Aceclofenac (10mg/kg, p.o)
Group III (Test 1): Hydro alcoholic extract of Rhizome of *Corallocarpus epigaeus*
(200mg/kg, p.o)
Group IV (Test 2): Hydro alcoholic extract of Rhizome of *Corallocarpus epigaeus* (400mg/kg, p.o)

Carrageenan induced rat paw edema

Carrageenan induced rat paw edema was done by the method of Winter et al. (1962).[121] Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The different groups of rats were administered with HAERCE (200 and 400 mg/kg, p.o.) and diclofenac (10 mg/kg, p.o.). The control group received vehicle (Distilled water, 10 l/kg, p.o.). 1 h after drug treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent). The paw volume was measured by a Plethysmometer. The measures were determined at 0 h (Vo: before edematogenic agent injection) and 1,2,3,4 and 5h intervals later (Vt). The difference between Vt and Vo was taken as the edema value. The percentage of inhibition was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{(Vt - Vo)\text{control} - (Vt - Vo)\text{treated}}{(Vt - Vo)\text{control}} \times 100$$

Experimental Design

Induction of arthritis

RA was induced according to the method previously described by Pearson [122]. Arthritis was induced intradermally by injection of 0.1 mL chicken type II collagen in CFA heat killed Mycobacterium tuberculosis and sterile paraffin oil (10 mg/mL] into the left hind paws of rats groups 2 to 5 according to their body weights. Treatment with Hydro alcoholic extract of Rhizome of *Corallocarpus epigaeus* (200mg/kg, p.o) started from the 10th day after arthritis induction and continued for 21 days. The treatment lasted for three weeks after which the animals were humanely immobilized by cervical dislocation. Thereafter, blood samples were collected by cardiac puncture into sterile bottles. The blood samples were centrifuged (3000 g for 15 min) and serum separated for biochemical analyses.

Experimental Groups

A total number of 20 Wistar albino rats divided into five groups of 4 rats each were used.
Group 1: Normal control received normal saline 5 ml/kg
Group 2: Negative control (untreated arthritic rats) received 5 mL/kg normal saline
Group 3: Positive control (arthritic rats) treated with Aspirin (100 mg/kg) standard drug Group 4: Arthritic rats treated with Hydro alcoholic extract of Rhizome of *Corallocarpus epigaeus* (200mg/kg, p.o)
Group 5: Arthritic rats treated with Hydro alcoholic extract of Rhizome of *Corallocarpus epigaeus* (400mg/kg, p.o)

Measurement of paw volume, joint diameter and arthritic score

The severity of arthritis was evaluated on day 7, 14 and 21. For this purpose, Plethysmometer (UGO Basile, Italy) was used to measure paw volume [123]. Digital Vernier caliper (Mitutoyo, Japan) was used to measure joint diameter [124]. For each animal, variation of edema, joint diameter, paw withdrawal latency responses (pain threshold) and paw withdrawal latencies (thermal hyperalgesia) were expressed as % values relative to the pre-administration value (100%) [125]. Evaluation of the degree of arthritis was assessed daily by visual observation. A score of 0–4 helped distinguish the different disease stages with a maximum value of 4 for each rat. Scores was attributed according to the parameters such as edema, erythema, malformation and incapacity to use the limb [126].

Measurement of Biochemical estimations

On day 22, after anesthesia (using anesthetic ether), cardiac puncture was used to draw blood and introduce into a tube containing EDTA as anticoagulant and into another tube without anticoagulant; Hematological parameters like erythrocytes (RBC) and leukocytes (WBC) counts, hemoglobin (Hb), Hematocrit and platelets (PLT) were determined in blood with anticoagulant by the usual standardized laboratory method [127]. Otherwise, blood without anticoagulant was centrifuged for 5 min (4900 rpm) and the serum was collected, then serum AST, ALT, ALP, total protein, C-reactive protein (CRP) and Rheumatoid factor (RF) levels was also quantified [128].

Measurement of proinflammatory cytokines

The blood of experimental animals was collected and serum was separated by clotting at room temperature for 30 min [129]. The protein concentration of serum proinflammatory cytokines such as TNF-a, IL-6, IL-1b and IL-10 was evaluated using ELISA kits and the procedure was carried out according to manufacturer's instructions.

Measurement of serum antioxidant and prooxidant activity

Malondialdehyde levels was estimated by the method of Devasagayam and Tarachand [130] in serum of arthritic rats.The colour intensity was read at 532nm and the result was expressed in nmol/mg protein. Antioxidant activities of superoxide dismutase (SOD), catalase and reduced glutathione was determined using standard protocols [131-133].

Histopathological study of joint

The interphalangeal joints were fixed in formol acetic acid solution and were later removed and cleaned with distilled water twice. The samples were soaked in graded 30, 50, 70, and 95%,ethanol each for 2 h and finally in absolute ethanol overnight for dehydration. The dehydrated samples were cleared by placing them in turn through 3:2, 1:1, and 1:3 volume/volume of absolute ethanol/xylene series and then through pure xylene for 3 h. Each of them was kept in molten wax at 50-60^{0}C for 48 h for infiltration of the wax into the tissues. The specimens were placed in the embedding mould and molten wax poured on them, and set aside to cool. Plastic block was then attached to the wax block containing the sample and to a rocking microtome for sectioning.

The cut sections were laid on slides daubed with albumin of an egg, cleared with graded ethanol - xylene solutions and dyed with hematoxylin and eosin. The slides were allowed to dry in an oven, thereafter microscopic examinations was done using light microscope and photomicrograph.

Statistical analysis

All the data were expressed as mean ± standard deviation. Continuous data were assessed for significant differences between means using the one way ANOVA test. A significance threshold of $p < 0.05$ was adopted for the analyses. Value of ($p < .05$) was viewed to be statistically significant.

RESULT

Pharmacognostic studies

Proximate analysis

Hydro alcoholic extract of rhizome of ***Corallocarpus epigaeus*** showed 3.8% *w/w* percentage yield. The physico-chemical parameters are helpful in judging the purity and quality of the drug. The percentage of active principles in the plant is determined only in the dry condition. Hence the parameters determined for proximate analysis include moisture content ,total solid content, ash value and extractive value content of the ***Corallocarpus epigaeus*** **extract**. Results of each parameter evaluated are given in Table 2. The present study of phytochemical analysis of ***Corallocarpus epigaeus*** has suggested the presence of various phytocompouds as shown in Table 3. Total flavonoids content (TFC) present in ***Corallocarpus epigaeus*** extracts as quercetin equivalent (QE), respectively; have been determined using spectrophotometer.

Amount of TFC present in ***Corallocarpus epigaeus*** extracts are given in Table 4.

Table 2: Physiochemical analysis of *Corallocarpus epigaeus* extracts

Parameter	C. epigaeus
Moisture content	0.64% w/w
Total solid content	98.15% w/w
Total ash	3.70% w/w
Acid insoluble ash	1.12% w/w

Table 3: Phytochemical screening of extracts of *Corallocarpus epigaeus*

Constituents	Pet.Ether extract	Hydroalcohol extract
Alkaloids	-	+
Glycosides	-	-
Flavonoids	-	+
Saponins	+	+
Phenolics	-	+
Amino Acids	+	-
Carbohydrate	+	+
Proteins	+	-

Table 4: Quantitative phytochemical analysis of hydroalcoholic extract of *Corallocarpus epigaeus*

Total Phenol (mg/g)	Flavonoid (mg/g)
62.33 ± 0.90	13.25 ± 0.28

Acute oral toxicity study

The acute oral toxicity study was performed according to OECD 423 guidelines. A single oral administration of a starting dose of 2000 mg/kg body weight, of hydroalcoholic extract of ***Corallocarpus epigaeus***.(HAECE) was administered to 3 male rats and observed. There was no lethality, mortality or any toxic reactions found at any selected dose level until the end of the study period. The results of acute oral toxicity studies are shown in Table 2.

Table 5: Acute Oral Toxicity Study of hydroalcoholic extract of *Corallocarpus epigaeus*.

S.no	Treatment	Dose	Weight gms	Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
1	HAECE	2000mg/kg	185	No signs of toxicity	Nil	Nil	14 days
2	HAECE	2000mg/kg	190	No signs of toxicity	Nil	Nil	14 days
3	HAECE	2000mg/kg	188	No signs of toxicity	Nil	Nil	14 days

IN-VIVO STUDY MEASUREMENT OF PAW EDEMA

Table 6: Effect of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus*

Group	Initial Paw Volume	Paw volume After Induction(ml) as measured by plethysmometer			
		1 hr	2 hr	4hr	6hr
I	1.195 ± 0.012	1.192 ± 0.010	1.190 ± 0.009	1.184 ± 0.007	1.199 ± 0.015
II	1.188 ± 0.010	2.012 ± 0.038	2.345 ± 0.058	2.545 ± 0.063	2.790 ± 0.124*a
III	1.193 ± 0.011	2.085 ± 0.037	1.758 ± 0.043	1.536 ± 0.044	1.245 ± 0.032*b
IV	1.184 ± 0.009	2.124 ± 0.048	1.805 ± 0.040	1.662 ± 0.037	1.302 ± 0.026*b
V	1.202 ± 0.016	2.228 ± 0.054	1.840 ± 0.047	1.587 ± 0.042	1.284 ± 0.028*b

Values are expressed as Mean±SEM
*a Values are significantly different from normal control
*b Values are significantly different from Toxic control

Corallocarpus epigaeus on carrageenan induced paw edema indicates that the change which occurs due to the treatment of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* in carrageenan induced paw edema by measuring the digital plethysmometer. (UGO Basile, Italy)

Percentage inhibition

Table 7: Effect of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus*.

Group	Initial Paw Volume	6 hr (ml)	Inhibition percentage
I	1.195 ± 0.012	1.199 ± 0.015	----
II	1.188 ± 0.010	2.790 ± 0.124*a	
III	1.193 ± 0.011	1.245 ± 0.032*b	55.37%
IV	1.184 ± 0.009	1.302 ± 0.026*b	53.33%
V	1.202 ± 0.016	1.284 ± 0.028*b	53.97%

Values are expressed as Mean±SEM
*a Values are significantly different from normal control
*b Values are significantly different from Toxic control

Percentage inhibition of carrageenan induced paw edema. It indicates that the change which occurs due to the treatment of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus*.

The hydroalcoholic extract of rhizome of ***Corallocarpus epigaeus*** inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the hydroalcoholic extract of rhizome of ***Corallocarpus epigaeus*** may possess arachidonate COX inhibitory property.

Table 8: Effect of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* on paw volume in FCA-induced arthritis rats

Groups	Treatment	Paw volume in (ml)		
		7th day	14th day	21st day
G1	Normal control 10ml/kg Normal saline	1.28±0.27	1.23 ± 0.24	1.36 ±0.33
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	3.44 ± 0.68	5.22 ± 0.84	5.90 ±0.94*a
G3	Standard control Aspirin 100mg/kg	3.68 ±0.72	4.12 ± 0.84	4.78 ±0.94*b
G4	Treatment control HAECE 200mg/kg	3.84 ±0.78	4.48 ± 0.88	5.12 ±0.98*b
G5	Treatment control HAECE 400mg/kg	3.73 ±0.75	4.59 ±0.93	4.88 ±0.96*b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01 *a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Fig 5: Normal Control

It shows no significant edema

Fig 6: Toxic
It shows edema in right hind paw of rat

Fig 7: Standard
It shows significant reduction of edema

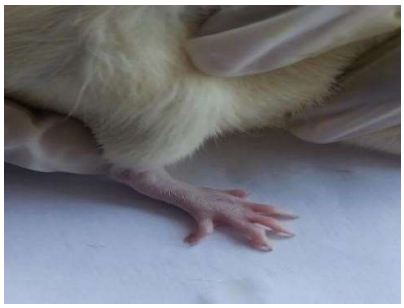

Fig 8: Treatment control (Low dose)
It shows mild edema

Fig 9: Treatment control (High Dose)
It not shown in edema

Table 9: Effect of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* on Joint diameter in FCA-induced arthritis rats

Groups	Treatment	Joint diameter (% Increase)		
		7th day	14th day	21st day
G1	Normal control 10ml/kg Normal saline			
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	98.3±3.54	104.2 ±4.35	113.8 ±4.86*a
G3	Standard control Aspirin100mg/kg	62.2 ±2.62	64.5 ±2.94	54.30±2.20*b
G4	Treatment control HAECE 200mg/kg	71.8 ±3.05	88.6 ±3.37	94.3 ±3.55*b
G5	Treatment control HAECE 400mg/kg	69.6 ±2.94	84.2±3.18	90.14 ±3.32*b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01
*a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Table 10: arthritic score index in FCA-induced arthritis rats

Groups	Treatment	Arthritic score index (ml)		
		7th day	14th day	21st day
G1	Normal control 10ml/kg Normal saline			
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	5.32 ± 0.87	11.45 ± 1.15	15.45 ±1.36*a
G3	Standard control Aspirin 100mg/kg	4.92±0.78	7.25 ± 1.02	10.30 ±1.24*b
G4	Treatment control HAECE 200mg/kg	4.42 ±0.70	7.94 ± 1.22	11.45 ±1.36*b
G5	Treatment control HAECE 400mg/kg	4.85 ±0.77	7.50 ±1.18	10.90 ±1.24*b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01
*a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Table: 11 Influence of the hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* on haematological parameters in CFA-induced arthritis in rats

S.no	Treatment	Haemoglobin (g/dl)	RBC (million/µl)	Hematocrit (%)	WBC (10^9/L)	Platelet (10^9/L)
G1	Normal control 10ml/kg Normal saline	13.95±1.08	7.62±0.83	39.70±1.90	8.10±0.75	803.00±37.50
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	8.70 ± 0.84*a	3.82 ± 0.24*a	25.30 ± 1.25*a	13.20 ±1.45*a	1730.00 ± 73.10 *a
G3	Standard control Aspirin 100mg/kg	12.90±1.02*b	6.88 ±0.73*b	36.10 ±1.72*b	11.30 ±1.08*b	1095.00 ±55.50 *b
G4	Treatment control HAECE 200mg/kg	10.80 ±0.93*b	5.90 ±0.64 *b	30.44±1.40*b	9.45 ±0.80*b	1320.00 ±62.30 *b
G5	Treatment control HAECE 400mg/kg	11.95 ±0.98*b	6.32 ±0.70 *b	33.60±1.53*b	10.60 ±0.93 *b	1210.00 ±58.65 *b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01
*a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Table 12: serum parameters in CFA-induced arthritis in rats

S.no	Treatment	CRP (mg/l)	RF (IU/ml)	ALP (U/l)	ALT (U/l)	AST (U/l)	Total protein (g/dl)
G1	Normal control 10ml/kg Normal saline	1.73±0.18		72.90±2.94	45.60±2.45	39.40±1.90	7.03±0.64
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	7.05 ± 0.74*a	61.00 ±2.78*a	455.70± 22.40 *a	174.60 ±4.30 *a	132.70 ± 4.15 *a	4.75 ± 0.43*a
G3	Standard control Aspirin 100mg/kg	4.10±0.38*b	38.35±1.90*b	195.40 ±6.20*b	102.90 ±3.05*b	88.10 ±2.45 *b	5.40 ±0.60 *b
G4	Treatment control HAECE 200mg/kg	5.45±0.63 *b	44.00 ±2.08*b	275.30 ±8.35*b	131.20 ±3.60*b	107.40 ±2.80 *b	6.05 ±0.72 *b
G5	Treatment control HAECE 400mg/kg	4.95 ±0.57 *b	41.70 ±1.96*b	222.10 ±6.50*b	120.80 ±3.35*b	96.4 ±2.58 *b	5.80±0.66*b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01 *a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Table 13: *Corallocarpus epigaeus* on the levels of TNF-a, IL-6, IL-10 and IL-1b in CFA-induced rheumatoid arthritis rats

S.no	Treatment	TNF-a (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	IL-1b (pg/ml)
G1	Normal control 10ml/kg Normal saline	52.25±1.45	134.70± .45*a	58.05±2.50	43.45±2.05
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	143.20±3.90*a	254.10±5.10*a	102.15± 3.75*a	84.10 ±2.75 *a
G3	Standard control	88.75±2.30*b	150.35±3.80*b	67.20 ±2.65*b	57.80 ±2.40*b

	Aspirin100mg/kg				
G4	Treatment control HAECE 200mg/kg	101.40±3.05*b	174.10±4.20*b	81.30 ±3.05*b	68.40 ±2.65*b
G5	Treatment control HAECE 400mg/kg	95.6 ±2.75*b	161.55±3.98*b	75.80 ±2.80*b	61.40 ±2.56*b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01
*a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Table 14 : **Effect of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* on oxidative stress in CFA-induced rheumatoid arthritis rats**

S.no	Treatment	MDA nmol/mg/ Protein	SOD (U/ protein)	ıg CAT (U/mg protein)	GSH (U/mg protein)
G1	Normal control 10ml/kg Normal saline	4.62±0.52	42.30 ± 1.65*a	18.70±1.02	61.60±2.08
G2	Arthritic control (0.1 mL chicken type II collagen in CFA)	8.78 ± 0.94*a	20.10 ± 1.20*a	9.15± 0.78*a	22.75 ±1.05*a
G3	Standard control Aspirin 100mg/kg	5.18±0.64*b	38.45± 1.48*b	15.50 ±0.94*b	50.40 ±1.95*b
G4	Treatment control HAECE 200mg/kg	6.22±0.78*b	31.05 ±1.33*b	11.65 ±0.80*b	41.70 ±1.42*b
G5	Treatment control HAECE 400mg/kg	5.66 ±0.72*b	36.00 ±1.40*b	13.75 ±0.88*b	47.05 ±1.64*b

The values represent the mean ± SEM and analyzed by one way ANOVA followed by Newmann keuls multiple range test. Values are significantly different at the P < 0.01 *a Values are significantly different from Normal control.
*b Values are significantly different from arthritic control

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on paw volume

Table 8 reveals that, hydroalcoholic extracts of *Corallocarpus epigaeus* treated groups significantly (P < 0.001) lowered the paw volume from the first day until the end of treatment as compared to arthritis control group. Indomethacin (10mg/kg) significantly (P < 0.001) reduced the increase of edema induced by CFA from the 3rd day till the last day of treatment.

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on joint diameter

Joint diameter of rats significantly (P < 0.001) increased in all rats on the groups treated with CFA. The hydroalcoholic extracts of *Corallocarpus epigaeus* (200& 400 mg/kg) considerably (P < 0.001) reduced the joint volume from the first day till the end of treatment as compared to arthritis control group. Indomethacin exhibited an important (P < 0.01) activity from the 5th day. (Table.no:9).

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on arthritic score

Morphological variation materialized by the arthritic score was significant (P < 0.001) in all animals that received a sub-plantar administration of CFA. The hydroalcoholic extracts of *Corallocarpus epigaeus at* both doses and indomethacin effectively protected the animals against the exaggeration of morphological variation observed in untreated animals; this was reflected by a significant variation (P < 0.001) of arthritic scores between animal treated groups and those of the untreated group (Table no:10).

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on hematological parameters

Table 11 shows the effects of the extracts on changes in hematological parameters 21 days after administration of the CFA. The results of this table reveal that, in animals of the untreated group, the levels of platelets and WBC are significantly increased (P < 0.001) while the levels of RBC, Hb and hematocrit are significantly decreased compared to animals of healthy group. Moreover, the results also show that, both doses of hydroalcoholic extracts of *Corallocarpus epigaeus* extracts or indomethacin significantly attenuated these changes in such a way that, at 400 mg/kg, there is no significant variation between animals of the healthy group and those of the groups treated with hydroalcoholic extracts of *Corallocarpus epigaeus*.

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on Biochemical parameters

The results in Table 12 show that, in untreated animals (arthritic control group), serum levels of CRP, RF, AST,ALT and ALP significantly increased ($P < 0.001$) and total protein level significantly decreased ($P < 0.001$) compared to the parameters of animals of the healthy group. In animals treated with both doses of hydroalcoholic extracts of *Corallocarpus epigaeus* or indomethacin,all biochemical parameters evaluated tend to return to normal values.

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on cytokines

Table 13 displays the serum levels of proinflammatory cytokines such as TNF-a, IL-6, IL1b and IL-10 in various experimental groups. Assessment of serum levels of cytokines in various experimental groups revealed that there was a significant increase in serum levels of TNF-a, IL-6, IL-1b and IL-10 in group II rats when compared with control animals.

Upon supplementation with both doses of hydroalcoholic extracts of *Corallocarpus epigaeus* and indomethacin, the serum levels of TNF-a, IL-6, IL-1b and IL-10 were markedly brought down in group III,IV and group V rats, when compared to the untreated counterparts.

Effect of hydroalcoholic extracts of *Corallocarpus epigaeus* on lipid peroxides and antioxidant status

Table 14 shows the impact of hydroalcoholic extracts of *Corallocarpus epigaeus* on the levels of MDA in serum of various experimental rats. Assessment of MDA levels in various experimental groups dictate that MDA levels were elevated in arthritis induced animals. On both doses of hydroalcoholic extracts of *Corallocarpus epigaeus* and Indomethacin supplementation, the MDA levels were markedly brought down in group III, IVand group V rats in comparison with CFA alone administered rats. Status of oxidative stress in CFA induced animals can be evaluated by measuring the levels of antioxidants. CFA induced animals exhibited marked decrease in levels of antioxidant enzymes when compared with control rats. Treatment groups showed marked improvement in the activities of antioxidant enzymes when compared with CFA alone administered rats.

Histology of ankle joints

Histopathology of the ankle joint of healthy control rats revealed no inflammation, a few lymphocytes infiltration and no bone necrosis. A massive influx of inflammatory cells, cartilage destruction, proliferation of granulation tissue, lymphocytes infiltration and chronic inflammation was detected in arthritic control. In contrast to these pathological changes, animals having received hydroalcoholic extracts of *Corallocarpus epigaeus* or indomethacin showed significant protection against necrosis of bones with low influx of inflammatory cells and minimal bone damage compared. (Fig. 10-14).

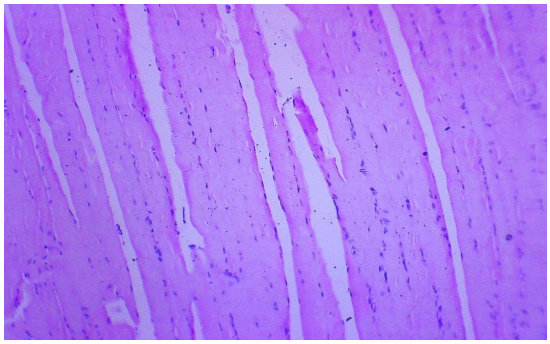

Fig 10: Normal control shows normal mature bony trabeculae with surrounding soft synovial and muscular tissue shows no significant pathology. There is no evidence of inflammation/ necrosis seen in the section studied.

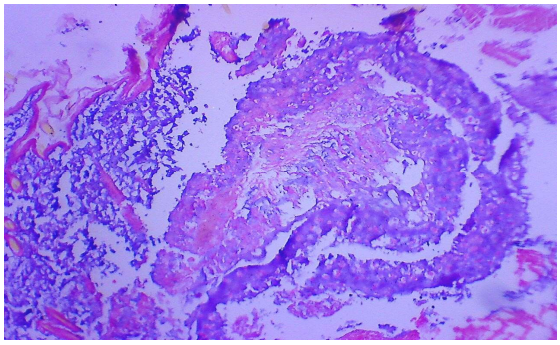

Fig 11: Arthritic control Section studied from joint shows bony tissue with focal necrotic areas and surrounding synovial tissue shows inflammatory infiltrates. Deep muscle shows scattered inflammatory infiltrates.

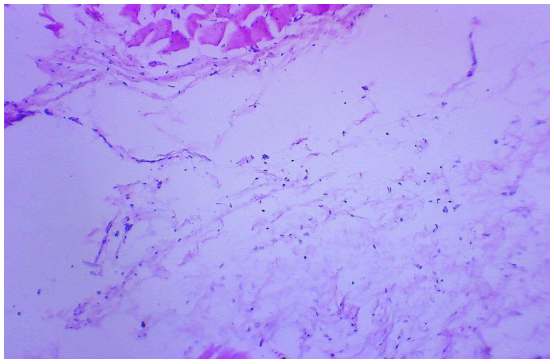

Fig 12: Standard control Section studied from the joint shows normal mature bony trabeculae separated by bone marrow spaces. Surrounding synovial tissue shows mild edematous with occasional scattered inflammatory infiltrates.

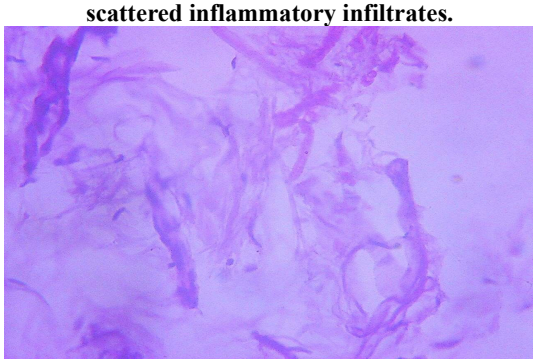

Fig 13: Treatment control (HAECE 200mg/kg) Section studied from joint shows mature bony trabeculae with scattered area shows necrotic bone. Surrounding stroma shows synovial tissue with mild edema. Deep muscle shows no significant pathology.

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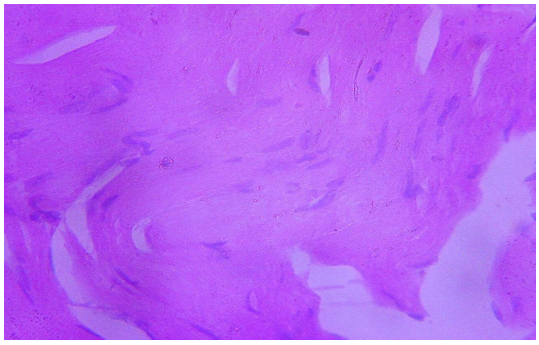

Fig 14: Treatment control (HAECE 400mg/kg) Section studied from joint shows normal mature bony trabeculae, surrounding synovial tissue shows no inflammatory infiltrates. Deep muscle shows no significant pathology.

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DISCUSSION

At present, most inflammatory diseases are treated with conventional anti-inflammatory drugs, such as steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs). However, prolonged use of these drugs may produce many adverse effects, including gastrointestinal disorders,[134,135] immunodeficiency and humoral disturbances.[136] From long time plants have been used as source of drugs for the treatment of various ailments in developed as well as developing countries.

Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min [137,138].

Carrageenan induced rat paw edema is a suitable *in vivo* model to predict the value of antiinflammatory agents, which act by inhibiting the mediators of acute inflammation [139]. Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270–360 min) is associated with the activation of kinin-like substances, i.e.,prostaglandins, proteases and lysosome [140,141]. In the present study, hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* was evaluated for its and antiinflammatory activity in experimental rats. Preliminary phytochemical analysis of the hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* showed the presence of phytochemical such as Alkaloids, Carbohydrate, Steroids, Phenols, and Flavanoids.

Acute oral toxicity studies of the hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* were performed by using OECD 423 guidelines. Studies did not exhibit any lethality or any profound toxic reactions at a dose of 2000 mg/kg/p.o. According to the OECD 423 guidelines, for acute oral toxicity study, LD50 dose of 2000 mg/kg/p.o of the hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* was found to be safe.

From this statistical analysis, it was concluded that the 'Carrageenan induced' Group II shows the inflammatory action and elevated level of Paw Volume is observed. But the 4th and 5th group deals with the anti-inflammatory activity of the hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* which gives low Paw Volume at the end of 6th h. Significant inhibition of paw edema was observed with both doses tested till the sixth hour. However, maximum inhibition of paw edema was found to be in Group IV and V and although the inhibition of paw edema with the extract was lesser than that found with the standard drug Indomethacin. The low percentage of inhibition is 43.12 % which belongs to the Group II i.e., Carrageenan alone induced. The duration of action was found to be comparable to that of Indomethacin till the sixth hour during investigation.

The result obtained from the study indicates that the hydroalcoholic extract of rhizome of ***Corallocarpus epigaeus*** (200 & 400 mg/kg) pretreatment possess significant protection against Carageenan induced paw edema.

Rheumatoid Arthritis, with symptoms such as swelling (joints), release of RF (autoantibody), deformity (bone destruction) and systemic change, is a more frequent disease that presents major systemic clinically complications with a high mortality rate in patients compared to healthy people [142]. In rheumatoid arthritis, swelling of the synovium due to the proliferation of synovial cells, is considered the main actor to affection and deterioration of cartilage with consequences such as the loss of the protective role of the synovial liquid by altering the binding properties of proteins in the cartilage [143]. Bone erosion, associated with increased and prolonged inflammation, affects 80% of patients and occurs rapidly [144,145].

The experimental model of polyarthritis induced by CFA on rat is widely used for preclinical testing of numerous anti arthritic agents [146]. This model, due to its close similarities with human rheumatoid diseases, is widely used to evaluate inflammatory disease and valid as a chronic pain model [147,148]. In the present study, hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* treatment showed antiarthritic potential in all the

inflammatory parameters. It significantly decreased the inflammation in treated animals by reducing the paw volume, joint diameter and arthritic score. In addition, the significant decrease on body weight observed on animals in the arthritis group was completely corrected by the both doses of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus*.

In arthritic patients, serum CRP, prototype biomarker of systemic inflammation for acute phase reactants with a level that increases rapidly during inflammatory processes, is used as a useful serum biomarker for evaluating the active inflammation [149,150]. Serum rheumatoid factor (RF) is an immunoglobulin molecule considered as "non self" capable of eliciting a reaction of the immune system [151].

In the pathogenesis of RA, abnormal changes in serum level of RF and CRP could be recognized as a strong indicator of RA [152]. CFA induced arthritis in rats increased the CRP and RF level as evidenced in the inflammatory process as shown by the results of the arthritis control group in this study. A significant ($P < 0.001$) decrease of RF and CRP level was observed after treatment with both doses of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus*.

To evaluate anti-arthritic activity of a drug, the level of AST ALT ALP and total protein provide an excellent and simple tool. Aminotransferases and ALP which are good indices of liver as kidney impairment,their activities significantly increased in adjuvant arthritis in rats [153]. In addition to the fact that the activity of serum alkaline phosphatase increased in pathogenicity of RA, the serum level of this enzyme like that of AST would play an important role in the release of biologically active compounds (bradykinins) in the inflammatory process [154, 155]. This enzyme being liberated into circulation during the bone formation and resorption, will be involved in localized bone loss as bone erosion and periarticular osteopenia [156].In addition, in about 30% of patients with RA, a significant elevation of serum ALT levels was observed [157]. In this study, arthritic rats showed significantly higher values of serum ALP, AST and ALT, while in animals with the different treatments (hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* or indomethacin), increased levels of these enzymes was significantly attenuated.

The factors which are closely associated with RA is found to disturb the state of equilibrium between proinflammatory and anti-inflammatory molecules [158]. Cytokines are the key factors that are involved in stages of inflammatory changes [159]. Cytokines are elevated markedly during initial phase of inflammation [160]. IL-12 and TNF-a were highly activated in inflammatory cells [161]. IL-6 plays a main role in RA pathogenesis in humans and in other associated inflammatory changes [162]. The observations of the study reported marked increase in cytokines levels in group II rats which is in correlation with earlier evidences. The hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* treatment has reduced the levels of these cytokines markedly. The results of the study correlate with literature evidences which shows that hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* has inhibited inflammation which is facilitated by TNF-a via ROS levels reduction [163].

Lipid peroxidation results from oxidative damage of membrane lipids which forms MDA [164]. Increased levels of MDA in group II rats has altered cellular membranes structure and function which eventually leads to increased ROS production [165]. Antioxidant property of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* has significantly decreased the MDA levels which correlates with the current results [166,167].

CFA administered rats also displayed marked decline in activity of antioxidant enzymes which might be due to accumulation of MDA that might have reduced the levels of CAT through inhibition of protein synthesis [168]. Glutathione depletion results in drastic metabolic effects which leads to increased sensitivity in cells [169]. hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* treatment significantly brought back the activities of antioxidant enzymes which proves the protective effect of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* as an antioxidant against arthritis [170].

In addition to this, arthritis was suppressed by hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* (200 & 400 mg/kg per day) treatment which was further confirmed by histopathological analysis. hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* treatment reduced the cellular infiltration, edema formation and inflammation in arthritis induced rats.Thus, the findings of the study propose that hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* might prevent the arthritis and may reduce the inflammation and joints destruction in arthritis induced animals.

CONCLUSION

The observation of the study substantiates that hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* was found to be protective against rats induced with CFA. It has reduced body weight, alleviate paw swelling, reduced, reduce arthritic score and decrease the inflammatory cytokines levels. The study similarly validated the promising antiarthritic activity of hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* which was assessed using biochemical, histopathological and molecular methodologies. The activity of the hydroalcoholic extract of rhizome of *Corallocarpus epigaeus* is strongly justified by its effect on the immune system and/or inhibitory properties to the release of pro-inflammatory mediators as observed in the in vivo study.

These results justify the use of this plant for decades in traditional treatment against inflammatory diseases including arthritis and classify this plant among the potential candidates for the isolation of novel anti-inflammatory and/or anti-arthritic products.

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