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(RESEARCH ARTICLE)

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Evaluation of anti-arthritic activity of *Hyptis suaveolens* seeds in rats

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Abstract

Rheumatoid arthritis has been treated with *Hyptis suaveolens* (L) poit. seeds. There has been no pharmaceutical assessment for rheumatoid arthritis. A study aims to assess the anti-arthritic properties of aqueous extract of *Hyptis suaveolens* (L) poit... seeds (AEHS). Standard techniques were used for phytochemical analysis. Tested anti-arthritic potential in-vitro (25-800µg/ml egg albumin) and in vivo (200 and 400 mg/kg egg albumin induced arthritic model). Chemicals such alkaloids, sugars, flavonoids, phenols, and terpenoids were found. Results showed 75% reduction of protein denaturation at 800µg/ml in-vitro. According to the Egg albumin model, AEHS considerably ($P < 0.0001$) inhibits changes in paw volume, joint diameter, and body weight. It also improves hematological, biochemical, and histopathological levels. The findings confirm the traditional usage of *Hyptis suaveolens*(L)poit. seeds to treat rheumatoid arthritis.

Keywords: *Hyptis suaveolens*; Rheumatoid arthritis; Protein denaturation; Egg albumin

1. Introduction

Plants cure all diseases. Plant treatments date back to human evolution. Few synthetic drugs existed 250 years ago. Plants provided most of the world's pharmaceuticals. Because they cannot afford or obtain allopathic treatments, 75% of the world uses these plants and other traditional products. To diagnose, prevent, and treat physical, mental, and social disorders, the traditional approach uses natural botanicals.

Plants inspire new pharmaceuticals because they boost human health. Medical plants are the richest bioresource for synthetic pharmaceuticals, nutraceuticals, food supplements, modern drugs, folk treatments, and pharmaceutical intermediates. To make complicated molecules, use plant chemicals. Individual and community health depends on medicinal plants as safer alternatives, therapeutic counterparts, or the only feasible treatment.

Medical plants are recommended by the WHO for variety. Human and community health depend on medicinal plant-based drugs' low side effects. As to WHO, 85% of traditional medicine in developing countries uses plant extracts. Some 3.5–4 billion individuals take plant-based drugs. Barks, flowers, roots, leaves, trunks, rhizomes, seeds, and fruits are botanical remedies. Latex, gum, and resins make several drugs.

Plant phytochemicals such tannins, sugars, alkaloids, terpenoids, phenolic compounds, steroids, and flavonoids have pharmacological effects. Plants create phytochemical substances through primary or secondary metabolism. Secondary metabolites are diverse with unclear functions. They are widely employed in agriculture, human therapy, veterinary research, etc. Many tribal cultures in India and China use legendary medicinal herbs to treat various illnesses. In many developing countries, the use and relevance of medicinal plants has spurred ethno medical data documentation.

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1.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) arises when the immune system attacks joints instead of bacteria and viruses. Joint discomfort and edema result from synovium thickening owing to inflammation. Joints move smoothly because to synovium fluid. Uncontrolled inflammation can damage bones and cartilage. Bone spacing decreases with cartilage loss. Joint discomfort, instability, and loss of movement might ensue. Deformities can affect joints. Due to its early onset and irreversibility, doctors recommend early diagnosis and thorough treatment for RA. RA usually affects the hands, feet, wrists, elbows, knees, and ankles. Most joint effects are symmetrical. One knee or hand usually affects the other. Systemic disorders like RA impact the heart and lungs. Body is systemic.

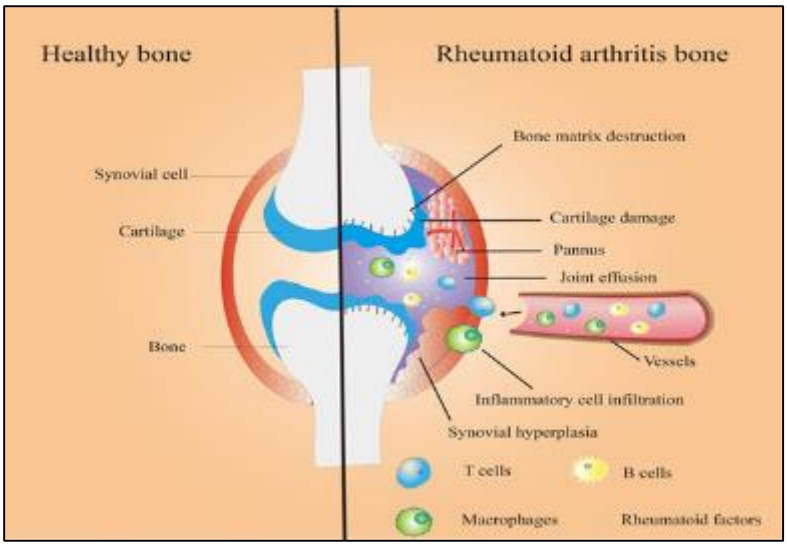

Figure 1 Rheumatoid Bone

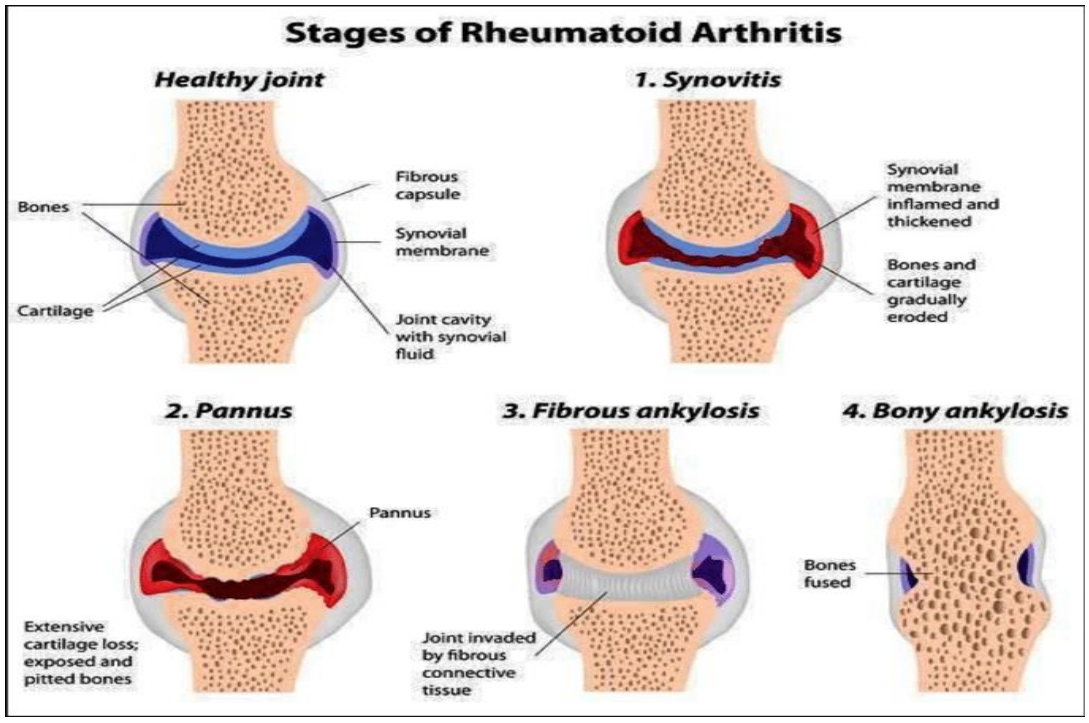

Figure 2 Stages of RA

1.1.1. Difference between Rheumatoid arthritis and Osteoarthritis

OA and RA can produce tight, aching joints that limit movement. OA doesn't cause inflammation or redness, but prolonged activity can cause edema. RA often damages both sides. If one arm or leg is affected, the same joint may affect the other. Clinicians use this to distinguish RA from osteoarthritis.

Nor is OA autoimmune like RA. Trauma or aging joints might cause it. OA usually affects seniors. Tennis players and other athletes who overuse a joint or have a catastrophic accident might also develop it. Autoimmune RA. RA joints are harmed by self-attack.

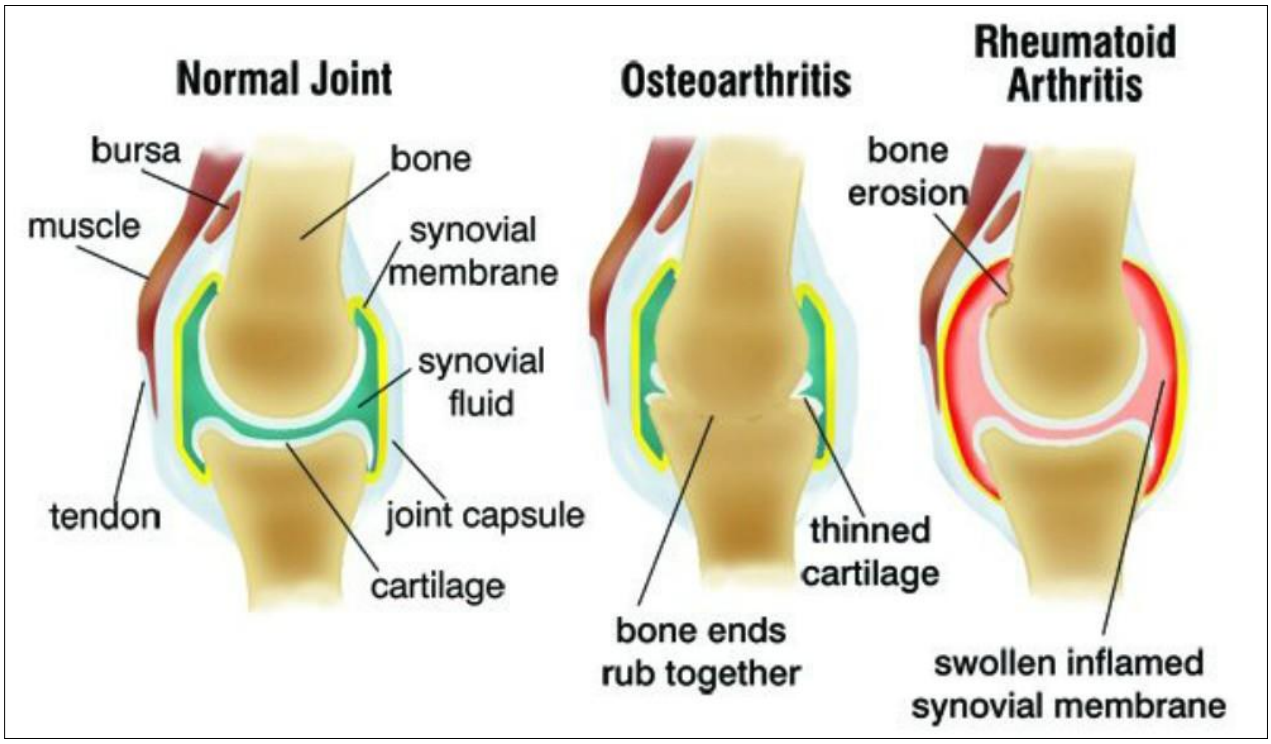

Figure 3 Rheumatoid Arthritis bone vs Osteoarthritis bone

1.2. Causes of RA

An aberrant immune system reaction causes RA's inflammation and joint degradation, say physicians. Genes, hormones, and environment may cause immune system issues, say scientists.

Patients with HLA shared epitopes have five times more RA. HLA manages immunity. RA is linked to STA4, which regulates and activates the immune system, TRAF1 and C5, which produce chronic inflammation, and PTPN22, which propagates RA. Genes do not cause RA in all.

Researchers look elsewhere. In individuals with susceptibility genes, female hormones (70 percent of RA patients are women), obesity, and stress reaction, bacteria or viruses can cause it. Environmental factors can induce rheumatoid arthritis. Air pollution, pesticides, smoking, workplace mineral oil and silica.

1.3. Symptoms of RA :

RA patients may initially have discomfort and pain but no redness or swelling in the joints.

The following joint symptoms indicate RA:

- Joint pain, tenderness, edema, or stiffness for six weeks or more
- Morning stiffness for 30 minutes or more
- Multiple joints affected
- Small joints (e.g., wrists, hands, feet) affected

- Same joints on both sides of the body affected
- Loss of range of motion
- Joint redness
- Rheumatoid nodules
- Joint warmth
- Joint deformity
- Weight loss
- Weakness

Many suffer from pain, weariness, loss of appetite, and low-grade fever. RA symptoms vary. Symptoms and inflammation rise during a flare. Flares last days or months. Symptoms disappear totally during remission. Continuing inflammation can harm the body. How RA affects organs and bodily systems:

- Eyes. Redness, discomfort, dryness, light sensitivity, and vision loss
- Mouth. Gum inflammation and dryness
- Skin. Rheumatoid nodules - tiny deposits under the skin over bones
- Lungs. Inflammation and scarring cause breathlessness.
- Blood vessels. Vascular inflammation can harm nerves, skin, and other organs.
- Blood. Anemia—low red blood cells.

1.4. Diagnosis of RA

Clinical signs may suggest RA to a primary care practitioner. The patient will be sent to a rheumatologist for RA diagnosis and treatment. Early RA symptoms may resemble other inflammatory disorders. Not one test can confirm RA. The rheumatologist will review medical history, perform a physical exam, and order tests to diagnosis.

1.4.1. Medical History

Doctors will ask about family medical history and current symptoms (pain, discomfort, stiffness, difficulty moving).

1.4.2. Physical Exam

Doctors will check joints for tenderness, swelling, warmth, and pain or limited movement. Affected joints' quantity and pattern can also indicate RA. Example: RA affects both sides of the body. Rheumatoid nodules or low-grade fever may be found on the exam.

1.4.3. Blood Tests

Inflammation and RA indicators like antibodies will be measured in the blood.

1.4.4. Inflammation

ESR and CRP are inflammatory indicators. High ESR or CRP is not specific to RA, however it helps diagnose RA when paired with antibodies.

1.4.5. Imaging Tests

An X-ray, ultrasound, or magnetic resonance imaging scan can detect joint deterioration such erosions and joint space constriction. If imaging studies show no joint injury, RA may still be present. It may indicate that the sickness is early and has not harmed the joints.

1.5. Treatment

- Rheumatoid Arthritis (RA) treatment aims to:
- Stop inflammation and enter remission
- Relieve symptoms and reduce complications
- Prevent joint and organ damage
- Enhance physical function and well-being

1.5.1. Strategies the doctor will use to achieve these goals:

Initial, vigorous treatment: Inflammation should be reduced or stopped as soon as feasible.

- Pursuing remission: Medical professionals call RA inflammatory disease activity. Ultimately, remission—minimal or no inflammation—is the goal. One method is "treat to target."
- Rigid control: "Tight control of RA" means keeping disease activity low. Researchers found that tight control slows joint degeneration.

Drugs for RA

Drugs treat rheumatoid arthritis. While some treat RA symptoms, others reduce or stop the illness and prevent structural damage.

- Drugs That Ease Symptoms

Over-the-counter and prescription NSAIDs are available.

- Drugs That Slow Disease Activity
    - **Corticosteroids:** Prednisone, Prednisolone and methylprednisolone are strong and fast-acting anti-inflammatory drugs.
    - **DMARDs:** DMARDs influence disease progression. Traditional DMARDs include methotrexate, hydroxycholorquine, sulfasalazine, cyclosporine, leflunomide, cyclophosphamide, minocycline and azathio Oral, self-injected, or doctor-infused medicines are available. DMARDs slow RA progression and protect joints and tissues by suppressing the immune system. Lifelong DMARD use is common. Early use is most effective, although benefits take 4–6 months. Side effects include liver damage, bone marrow suppression, and serious lung infections.
    - **Biologics:** DMARDs include these injections or infusions of biologics such TNF-alpha inhibitors may function faster than regular DMARDs. Some RA medications destroy the immune response, but they target specific steps in the inflammatory process. Biologics can halt, modify, or stop RA in many patients when other treatments fail. Pain, morning stiffness, and swollen joints can be reduced. Patients frequently see improvement after 2 weeks. Examples: infliximab, adalimumab.

- Surgery

RA surgery may never be needed, but it can be a vital choice for patients with irreversible damage that impairs functions, mobility, and independence. In severely damaged RA joints, joint replacement surgery can reduce discomfort and restore function. Metal and plastic are used to repair joints. Hip and knee replacements dominate. Replacement may be considered for ankles, shoulders, wrists, elbows, and other joints.

1.6. Risk factors

Sex, Age, Family history, Smoking, Environmental exposure, Obesity

1.7. Complications

Osteoporosis. Rheumatoid nodules, Dry eyes and mouth. , Susceptibility to infections. , Abnormal body composition, Carpal tunnel syndrome, Heart problems.. Lung disease, Lymphoma, Tendon rupture,Cervical myelopathy, Vasculitis

1.8. Plant profile

India grows many *Hyptis suaveolens*. The plant can be harvested in huge quantities from the wild and from Indian crops. Indians termed it "Chan/Wilaiti tulsi" and the morning soup made with corn was called "Bate"—memory help. Gastrointestinal secretions degrade its fragrant phytoconstituents, however mucilaginousness can be enhanced. H. Suaveolens root tea purifies blood and treats women's "diseases". Many Asian countries use it as a medicinal tea, South America uses it as food and essential oil.

1.8.1. Plant taxonomy

- Subkingdom – Tracheobionta
- Superkingdom – Spermatophyta
- Division – Magnoliophyte
- Class – Magnoliopsida
- Subclass – Asteridae

- Order – Lamiales
- Family – Lamiaceae
- Genus – *Hyptis Jacq*

Figure 4 *Hyptis suaveolens* plant Species –*Hyptis suaveolens*(L.)poit

1.8.2. Distribution

Pathside and wasteland weeds are Lamiaceae or Labiatae. An annual, perennial, subshrub, vine, or plant, pignut (H.suaveolens) Polluted roadsides, railways, wastelands, streams, pastures, and deciduous forests host this tropical American annual. No growth area is immune to thickets. Queensland, China, Indonesia, Papua New Guinea, Solomon Islands (Northern Territory), French Polynesia, Chuuk and the Icelandic Federal States (Yap Islands), Niue Islands, Guamand, and the Hawaiian Isles were infected4. West and Central African countries consider it a pest. The northern Indian Vindhyansk Forest between the Gangetic Plains and Narmadavalley is losing hyptisis.

1.8.3. Morphology

This scented herb reproduces by strapping seeds. Hairy, sticky stalk. Hyptis, a fragrant herb with square hairy tumors and orbicular to obviate leaven, grows to 2 m. Thicker, hairy leaf bottoms and serrulated edges. Three-cm smallpox. Branching flowers end in leaf buds in tiny cymes. The 5mm calyx features a ribbed blue corolla and 10mm fruit. The container's end has 1.21.5 mm fruit or seed nuts. Water, livestock, and automobiles distribute seeds. Many pollinators visit this huge seed. The seed can remain dormant for years and bloom vigorously from rootstocks after rain. The morphology mimics Ocimum organisms.

1.8.4. Cultivation:

The weed often forms large thickets that birds visit. When the seeds are ready, it starts flowering at 8-12 weeks, generating lots of seed that can be distributed by air, water, animals, and humans.

1.8.5. Seeds

Pignut or chan, Hyptis suaveolen seeds, are used in Mexico and Taiwan for drinking. Like psyllium seeds, it swells in water.[17]

Figure 5 Seeds

Figure 6 Swelled seeds

Swelling characters of seeds

In water, *Hyptis suaveolens* seeds grow 30 times their height, generating a thick mucilage covering.

Common names

Horehound, Pignut, Wild spikenard, Gros Baume, Wilaititulsi (Hindi), bhustrena, darptulas, junglitulas(Marathi), Tulasi (Telugu), bilatitulasi(Bengali), Ganga Tulasi (Oriya), bhustrena(Sanskrit).

Table 1 Proximate analysis of leaves of *Hyptis suaveolens*

Component partanalysis	**% Composition (c)leaves**	**% Composition (n)leaves**	**% Composition (t)leaves**
Protein	11.25	12.30	10.00
Lipids	4.20	3.00	2.00
Fibre	9.50	7.00	5.15
Carbohydrates	75.05	77.70	72.60
Moisture	80.75	83.53	82.75
Ash	12.35	18.35	11.40

Table 2 Phyto constituents

Diterpenes	**Suaveoli cacid,Suaveolol, Methylsuaveolate**
Steroids	β–sitosterol,β-sitosterolglycoside
Phenolic	Rosamarinicacid,Methylrosmarinate
Pleasingaroma	α– pinene
Others	Oleanolicacid,Oleanicacid,Ursolicacid, α–phellandrene

1.9. Physicochemical properties of extracted oil of *Hyptis suaveolens*

Parameters	*Hyptis suaveolens*
State(atroomtemperature)	Liquid
Colour	Paleyellow
Odour	Agreeable
Refractiveindex(at40^{0}C)	1.4319
Specific gravity(at25^{0}C)	0.8966
Acidvalue(mgKOH/g)	3.3
Iodinevalue	115.8
Unsaponifiablematter/w	0.68
Saponificationvalue(mgKOH/g)	195.0

GC-MS analysis was performed on hydro-distilled *Hyptis suaveolens* essential oils. Sabinene, limonene, bicyclogermacrene, beta-phellandrene, 1, 8-cineole, eugenol, beta-caryophyllene, beta-pinene, terpinolene, and 4-terpinol were the main ingredients.

1.10. Ethnobotanical uses

Tumor, Malaria, Headache, cancer, expectorant, fever, stomach ache, cold, yellow fever, Rheumatism, Analgesic, Constipation, Urethritis, Liver stimulant, Antisudorific, Depurative, Stomachic, Aperitifs, Dyspepsia, menorrhagia.

1.11. Medicinal uses

1.11.1. Appetizer

Anti-fungal Carminative Febrifuge Stomachic Flatulence Fever with cold Dermatitis Eczema

1.11.2. Boils Headaches

Poulatic of pounded fresh materials on snake bites Sores, dry and flaky skin

Essential oils have insecticidal activity Have better anti inflammatory activity

1.12. Pharmacological activities

Hyptis suaveolens' essential oil, alkaloid, flavonoid, phenol, saponin, flavorings, and sterols have medical effects, but its biological properties are unknown. The stimulant, carminative wound vine, sudorific, galactagogue, catarrhal disorder, and parasite skin illnesses are utilized in conventional medicine.

Anthelmintic added the leaves. They kill insects with their strong scent. Sapled H.suaveolens. This leaf is used on the head for headaches or tomato boils. For stomach pain, Sierra Leoneans consume Suaveolens.

1.12.1. Antimicrobial activity

Hyptis suaveolean leaves had broad in vitro antibacterial action against Fusariumoxysporum, Aspergillus niger, Helminthosporiumoryzae, Bacillus substilis, Staphylococcus aureus, E. coli, P. aeruginosa, and Micrococcus luteus.

1.12.2. Anti-inflammatory

Hyptis suaveolean exhibits potential topical anti-inflammatory effect more than indomethacin.

WOUND HEALING *Hyptis suaveolens* increases strength, granuloma breaking strength, wound contraction, hydroxyprolines, drygranulomas, and epithelization time. The plant's free radical cavities and granuloma tissue's antioxidants may boost wound healing.

1.12.3. Anti-oxidant activity

As an antioxidant, *Hyptis suaveolens* has catalase and superoxidedismutase.12 Granuloma tissue was tested for collagen lay-down pattern by Van Gieson and Masson Trichrome stains. High antioxidant enzyme values have been recorded.

1.12.4. Antiplasmodial activity

Hyptis suaveolens commonly used in traditional medicines for malarial medication and increased interest9.(14) (*Hyptis suaveolens*(L.) Poit excluded dehydroabietic. Plasmodium falciparum developed in vitro erythrocytes (IC50 26 – 27 μM) has been found to inhibit the production of both chloroquine-sensitive and chloroquine- resistant strains.

1.12.5. Antiulcer activity and gastro-protectiveactivity

The *Hyptis suaveolens* aqueous extract demonstrated strong effectiveness as an ethanolic extract, indicating that the plant promotes duodenal ulceration healing and inhibits its formation in rats.

1.12.6. Antifertility activity

Hyptis suaveolen extracts show anti-fertility effects in pregnant rats. Hyptis suaveolen leaf alcoholic extracts were 100% anti-fertility.

1.13. Plan of work

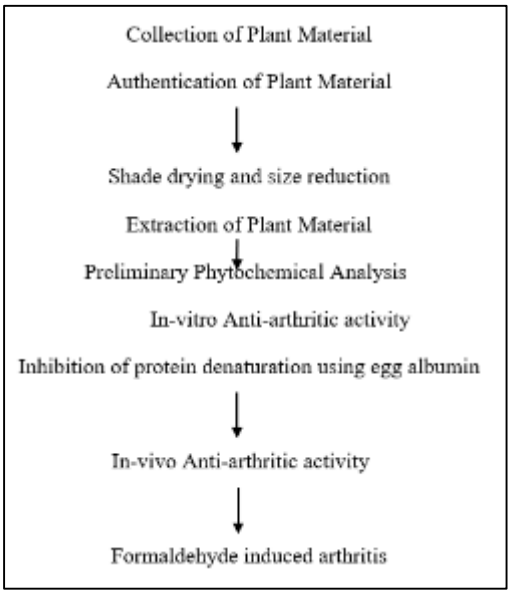

Figure.7 Plan of work

2. Materials and methods

2.1. Procurement of plant material

For the present investigation, seeds of *Hyptis suaveolens* seeds were purchased from the local market in Naraspur, Telangana.

2.2. Extraction process

Extraction is a way to separate medicinally active portions of plant or animal tissues from the inactive or inert components. The mixture is brought into contact with selective solvents used in standard extraction procedures.The procedure of exyraction used for *Hyptis suaveolens* is homoginiser. The seeds are soaked in water for 14 hours and the soaked seeds are subjected to homoginising using an ultrtorax homoginiser at 16,000 rpm for 2 hours and the mucilage is collected by the process of filteration using an muslin cloth and the extracted mucilage is placed in hot air oven for 7 days at 40o C until the extracted mucilage is collected in powdered form

2.3. General Methods of Extraction of Medicinal Plants

- Maceration
- Infusion
- Digestion
- Decoction
- Percolation
- Hot continuous extraction (soxhlation)

2.3.1. Soxhlation

Franz von Soxhlet devised the Soxhlet extractor in 1879. It was created to extract lipids from solids. Most often, Soxhlet extraction is utilized when the desired product has limited solvent solubility and the contaminant is insoluble. This allows unmonitored activity and recycles a tiny amount of solvent to dissolve more material.

Procedure

- The laboratory apparatus includes a flask, soxhlet extractor, and reflux condenser.
- For extraction, the raw material is placed in a filter paper thimble and inserted into the wide central tube.
- Alternatively, the medicine can be infused with menstruum and packed into the extractor, ensuring the bottom output is not blocked.
- Solvent boils in the flask.
- Vapor rises up the larger right-hand tube into the upper drug and condenses in the condenser before falling back onto the medication.
- Percolation extracts soluble components.
- When the extracts reach the top of the syphon tube, all percolates flow into the flask.
- Continue the process until the medication is fully extracted, then process the flask extract.
- Large volumes of medication can be extracted with less solvent.

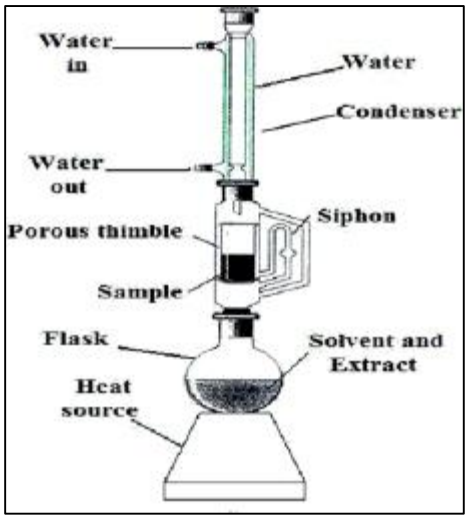

Figure 8 Soxhlet Extractor

2.3.2. Maceration

"The process in which properly comminuted drug is placed or permitted to soak in a solvent for specific period of time until the cellular structure is softened and penetrated by the solvent and soluble constituents are dissolved and extracted out".

Maceration Process

Following steps are involved in the general maceration process of extraction:

- The Plant material is crushed or cut into small pieces or made as moderately coarse powder.
- The crushed material is placed in a closed vessel.
- To the vessel, whole of the selected solvent (menstrum) is added.

- It is allowed to stand for three days (72hrs), by shaking occasionally.
- The liquid is strained off and the solid residue (marc) is pressed (to recover as much as occluded solution).
- The strained and expressed liquids are mixed.
- The obtained liquid is clarified by subsidence or filtration.
- The extract is then subjected for evaporation and concentration.

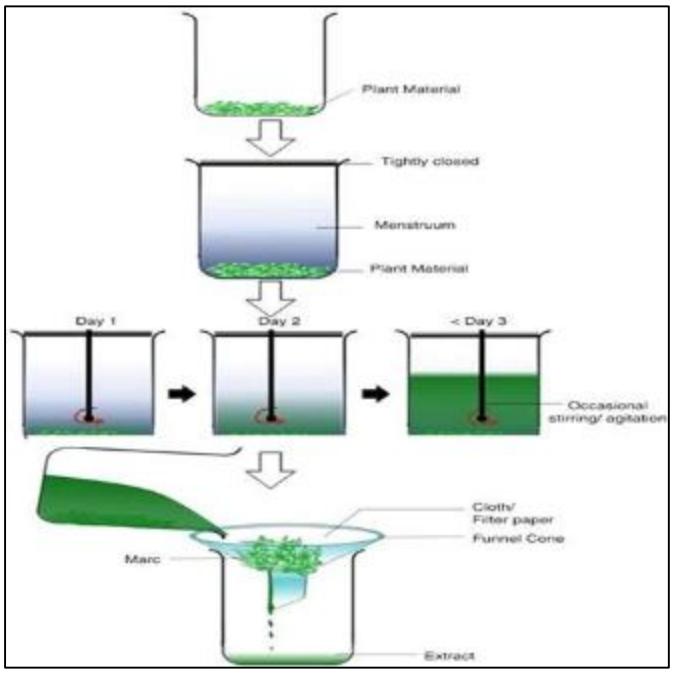

Figure 9 Maceration Process

2.4. Preliminary phytochemical analysis

Qualitative screening of *HYPTIS SUAVEOLENS* seed aqueous extract identified active chemical component classes. Table 7.1 shows preliminary phytochemical study results. Alkaloids, unsaturated sterols, saponins, glycosides, phenolics, terpenoids, tannins, flavonoids, carbohydrates, and proteins were found in phytochemical research. Preliminary phytochemical testing help identify plant chemicals.

2.4.1. Test for alkaloids

- **Mayer's Test**: The extract to be tested was treated with few drops of dilute 2N HCl and0.5 ml Mayer's reagent.White precipitate was obtained which confirms the presence of alkaloids.
- **Wagner's Test:** The extract was treated with few drops of 2N HCl and 0.5 ml Wagner's reagent. Brown flocculent precipitate was obtained which confirms the presence of alkaloids.
- **Hagers's Test**: The extract was treated with few drops of 2N HCl and 0.5 ml Hager's reagent. Yellow colored precipitate was obtained which confirms the presence of alkaloids.
- **Dragendroff's Test:**Dragendroff's reagent (potassium bismuth iodide solution) was added to the sample extract. The precipitate was reddish brown.

2.4.2. Test for carbohydrates

- **Molisch's test:** It was performed for the presence of carbohydrates. 1 ml of 10% alcoholic solution of alpha-napthol was added to the extracts and mixed. Then 1 ml of concentrated sulphuric acid was carefully poured along the sides of the test tube violet ring formed at the junction which was considered positive test for carbohydrates.

2.4.3. Test for reducing sugars

- **Fehling's test:** 5 ml of solution of extract was heated with equal volumes of Fehling's solution A & B. Transition of color from blue through green to reddish orange confirms the presence of reducing sugars.

- **Benedict's test:** 5 ml of solution of the extract was heated with 5 ml of Benedict's reagent. A green, yellow or orange red precipitate was considered as a positive test for reducing sugars.

2.4.4. Test for steroids

- **Salkowski reaction:** To 2 ml of solution of extract, 2 ml of chloroform and 2 ml concentrated sulphuric acid was added. Shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence. This confirms the presence of steroids.
- **Liebermann-**Burchard reaction: Mix 2 ml extract solution with chloroform and then 1-2 ml acetic anhydride and 2 drops of conc. sulphuric acid was added from the side of test tube. This confirms the presence of steroids.

2.4.5. Test for saponins

- **Foam's test:** A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was considered as a positive test for saponins.
- **Sodium Bicarbonate Test:** To the few milligrams of extract, few drops of sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

2.4.6. Test for tannins

- A small portion of extract was treated with 5 % ferric chloride solution. Appearance of green to blue color was taken as a positive test for tannins.
- Small portion of extract was treated with lead acetate. Appearance of creamy precipitate was considered as a positive test for tannins.

2.4.7. Test for flavonoids

- **Alkaline Reagent test:** The extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on further addition of dilute acid, was considered as positive test for flavonoids.
- **Lead Acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate confirms the presence of flavonoids.
- **Ferric chloride test:** A few drops of ferric chloride solution were added to the extract solution. Development of intense green color confirms the presence of flavonoids

2.4.8. Test for phenols

- **Ferric chloride test:** The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color confirms the presence of phenols.
- **Lead acetate test:** The extract was treated with 3 ml of 10% lead acetate solution. A bulky white precipitate confirms the presence of phenolic compounds.

2.4.9. Test for terpenoids

- **Salkowski test:** 2 ml of chloroform extract solution was carefully added to 3 ml of concentrated sulphuric acid to form a layer. A reddish brown colouration of the interface confirms the presence of terpenoids.

2.4.10. Test for glycosides

- **Keller –** Killani test: The extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. To the mixture, 1 ml of concentrated sulphuric acid was added. Appearance of brown ring at the interface indicates the deoxysugar characteristic of Cardenolides. Appearance of violetring below the brown ring and a greenish ring in the acetic acid layer confirms the presence of Cardiac glycosides.

2.4.11. Test for proteins

- **Millons test:** A small quantity of alcoholic extract is heated with Millons reagent. No white precipitate turned red on heating, confirms the presence of proteins.
- **Biuret test:** To one portion of alcoholic extract, 1ml of 10% sodium hydroxide solution is added, followed by 1 drop of dilute copper sulphate solution. Violet color is obtained which confirms the presence of proteins.
- **Ninhydrin test:** 2 drops of freshly prepared 0.2% ninhydrin reagent were added to the extract solution and heated. Development of blue color, confirms the presence of proteins.

2.4.12. Test for fixed oils and fats

Small quantities of extracts were pressed between two filter papers. An oily stain on the filter paper indicates the presence of fixed oils and fats.

2.5. Anti-arthritic activity in-vitro activity

Phytis suaveolens inhibits egg albumin denaturation for anti-arthritic properties.

In a 5 ml reaction mixture, egg albumin (0.2 ml), phosphate buffered saline (pH 6.4), *Hyptis suaveolens* seed extract (two ml), and diclofenac sodium (12.5-800 µg/ml) were present. Controlled double-distilled water volume. In a Biochemical oxygen demand (BOD) incubator, mixtures were heated at 70 °C for 5 minutes after 15 minutes at 37 ± 2 °C. Their absorbance was 660 nm. Protein denaturation inhibition formula:

% inhibition = (Abs control – Abs test sample / Abs control) * 100 Abs = Absorbance.

2.6. *In-vivo* activity

Evaluation of anti-arthritic activity of *Hyptis suaveolens* seeds against formaldehyde induced arthritis in rats [71,72]

2.6.1. Animals

Experiments on Animals were carried out at Vishnu Institute of Pharmaceutical Education & Research, Vishnupur, Narsapur, Medak Telangana 502313 India. CCSEA Registration No 1358/PO/Re/S/10/CCSEA35 Institutional Animal Ethics Committee gave its approval to all of the animal experiment protocols (proposalno. 05/IAEC/VIPER/Pharm/I/2024).Healthy adult male wistar rats weighing 250-300 gm were selected for the study. The animals were acclimatized to standard laboratory condition with temperature 25 ± 2ºC and fed with standard animal pellet feed (Hindustan lever limited) and water ad libitum.

Preparation of dose for extracts

Ethanolic extract (125 mg/kg,250 mg/kg & 500 mg/kg) of *Hyptis suaveolens* seed was formulated as suspension using 2% Tween-80 as solvent. The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

2.6.2. Material and Methods

Chemicals: Formaldehyde solution, fresh egg albumin, diclofenac sodium, 2% tween 80, *Hyptis suaveolens* seed aqueous extract (AEHS)

Treatment Design

- Group I : Normal control (normal saline)
- Group II : Rats received AEHS (125 mg/kg) + 0.1 ml 2% formaldehyde solution
- Group III: Rats received AEHS (250 mg/kg) + 0.1 ml 2% formaldehyde solution
- Group IV: Rats received AEHS (500 mg/kg) + 0.1 ml 2% formaldehyde solution
- Group V: Rats received aspirin (100 mg/kg) + 0.1 ml 2% formaldehyde solution

2.7. Anti-arthritic activity

Five six-animal groups were formed. Group I received normal saline, Group II-IV received AEHS (125,250,500mg/kg), and Group V received aspirin (100 mg/kg). Edema from formaldehyde was used to evaluate the in vivo anti-arthritic investigation. A sub plantar injection of 0.1ml 2% formaldehyde solution elicited chronic non-immunological arthritis 30 min after drug administration on day 1 and repeated on day 3. Using a micrometer screw gauge, the mean paw diameter increase over 10 days was used to diagnose arthritis.

Calculated left paw edema inhibition percentage

% inhibition= (VC-VT) X 100 / VC

Where VC= paw edema of control group , VT= paw edema of the test group

2.7.1. Parameters

Paw Volume

A water displacement plethysmometer measured paw volume. This was done before sub plantar formaldehyde injection (day 0) and every other day for 10 days afterward. Paw volume data was reported as % growth from day 0. Paw volume increase percentage formula:

% Increase in paw volume = (T0 – Tt / T0) * 100

Where To is the mean of paw thickness at day 0 and Tt is the mean of paw thickness at a particular time.

Blood analysis

After anesthesia with anesthetic ether, cardiac puncture was utilized to take blood into EDTA-containing and EDTA-free tubes on day 10.

- **Biochemical Parameters** Serum was obtained from anticoagulant-free blood centrifuged for 5 min (4900 rpm). Serum samples were analyzed for biochemical parameters as ALP, AST, ALT, RF, and CRP using standard kits in the fully automatic biochemical analyzer.
- **Hematological Parameters** The typical standardized laboratory procedure was used to measure hemoglobin (Hb), total leukocyte (WBC) and erythrocyte (RBC) counts, ESR, and PLT in blood with anticoagulant.
- **Histopathological studies** Cervical dislocation killed the animals on day 11. The hind paw ankle joints were dissected, weighed, submerged in 10% buffered formalin for 24 hours, decalcified in 5% formic acid, and paraffin embedded at 5 μm thickness. The sections were stained with haematoxylin and eosin and examined under a light microscope for synovium hyperplasia, pannus development, and joint space damage.

3. Results

Preliminary phytochemical analysis of Hyptissuaveolens(L)poit

The phytochemical screening of aqueous extract of Hyptissuaveolens(L)poit.. (AEHS) revealed the presence of carbohydrates, alkaloids, glycosides, flavonoids, aldose and ketose sugars, fats, phenolic compounds, amino acids and tannins

Table 3 Phytoconstituents present in AEHS

Phytoconstituents	**AEHS**
Alkaloids	+
Glycosides	+
Saponins	+
Steroids	+
Flavonoids	+
Phenols	+
Tannins	+
Fats	-
Proteins and amino acids	+

Note :+ Indicates present,- Indicates absent

ACUTE TOXICITY STUDIES Hyptissuaveolens in acute toxicity studies did not produce any symptom of toxicity and was observed no lethality up to dose of 2000 mg/kg body weight in rats. Hence, the extract was considered to be safe and non-toxic for further pharmacological screening.

In formaldehyde induced model, rats developed a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage, bone destruction and remodeling

3.1. Anti-arthritic activity

3.1.1. In-vitro anti arthritic activity

Hyptissuaveolens(L)poit…..at several doses (12.5-800ug/ml) provided considerable protection against denaturation of egg albumin. The results are summed up in the following table and its graphical representation is shown in the figure . The results show that extract produced 76.85% inhibition of protein denaturation at 800ug/ml. while diclofenac sodium brought about 83.75% suppression of protein denaturation at 800ug/ml

Table 4 Effect of AEHS on denaturation of Egg albumin

Concentration(µg/ml)	% of inhibitiondiclofenacsodium	% of inhibitionAEHS
25	11.30± 1.70	15.00± 5.50
50	18.30± 2.90	21.26± 8.45
100	26.20± 1.35	35.40± 1.45
200	46.18± 1.05	50.90± 2.65
400	71.00± 1.56	63.15± 1.56
800	83.75± 2.45	76.80± 1.60

3.1.2. % Inhibition Using Protein Denaturation Method

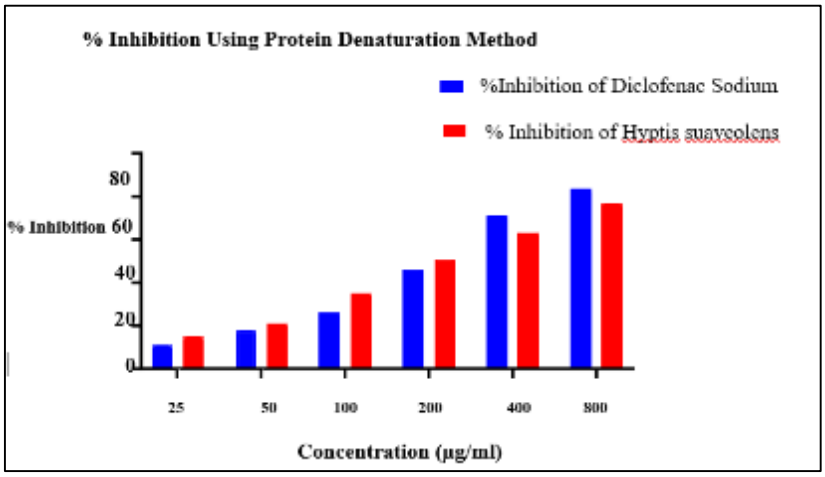

Figure 10 Effect of AEHS on denaturation of Egg albumin

3.2. Formaldehyde induced arthritis

Table 5 Effect of AEHS on Rat Paw diameter

Treatment	Day2	Day4	Day6	Day8	Day10	% inhibition
Control	0.448±0.016	0.614±0.012	0.824±0.004	1.08±0.007	1.32±0.002	-
AEHS(125 MG/KG)	0.36±0.016**	0.45±0.013**	0.60±0.012**	0.52±0.006**	0.48±0.006**	61.88%
AEHS(250 MG/KG)	0.368±0.012**	0.42±0.008**	0.52±0.004**	0.50±0.006**	0.42±0.012**	66.67%
AEHS(500 MG/KG)	0.224±0.018**	0.290±0.012*	0.24±0.025**	0.25±0.014**	0.12±0.009**	90.91%
Aspirin	0.264±0.011**	0.332±0.014*	0.32±0.011**	0.26±0.012**	0.19±0.016**	85.61%

The mean + SEM (n=6) was used.ONE-WAY ANOVA and dunnet's test were used to compare normal, illness, and standard controls. Significant values were evaluated between control group (a=p<0.01), disease control (p<0.01, p<0.05), and standard (A=P<0.01, B=P<0.05).

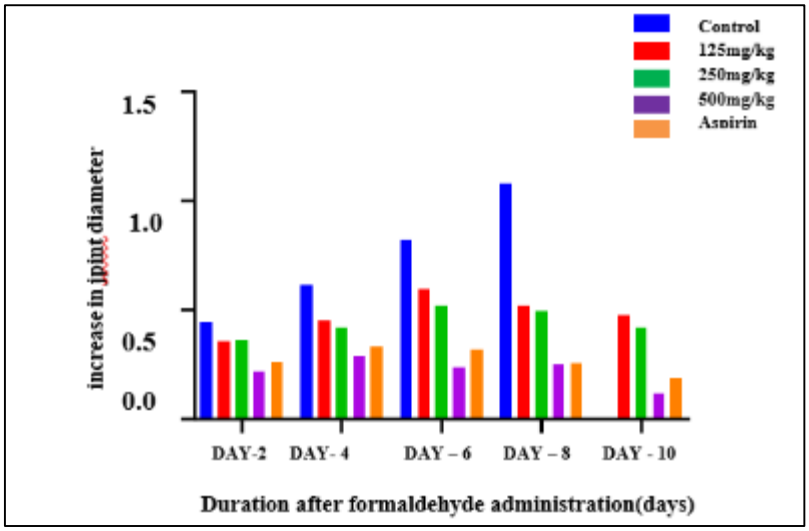

Figure 11 Effect of AEHS on Rat Paw diameter

3.3. Effect on formaldehyde induced arthritis

In formaldehyde induced arthritic model of wistar rats, the assessment made on 10 day showed that, treatment with different doses of AEHS (125,250,500 mg/kg p.o) aqueous extract has significantly reduced ($p<0.001$) swelling in the injected paw as compared to aspirin treated group. On the 10th day the % inhibition of paw edema exhibited by different dose of AEHS were 61.88%, 66.67%, 90.91%, respectively. While aspirin treated animals showed maximum inhibition of paw edema that is 85.61%.

Table 6 Effect of AEHS on hematological parameters

Parameters	Control	Aspirin	AEHS (125 mg/kg)	AEHS (250 mg/kg)	AEHS (500 mg/kg)
Haemoglobin(gm%)	15.9±0.08	15.3±0.08	10.4±0.05	13.1±0.07	11.1±0.06
TotalRBCcount(millions/mm^3)	8.00±0.044	8.72±0.04	5.64±0.03	8.54±0.04	7.89±0.04
Totalleucocytecount(cells/mm^3)	14.42±0.07	11.20±0.06	21.69±0.11	11.80±0.059	10.50±0.05
Neutrophils(%)	28±0.14	25±0.13	47±0.23	21±0.11	27±0.14
Lymphocytes(%)	61±0.31	59±0.30	86±0.43	65±0.33	68±0.35
Eosinophils(%)	02±0.01	0.3±0.02	05±0.02	04±0.02	05±0.03
Monocytes(%)	02±0.01	01±0.01	04±0.02	02±0.01	03±0.02
Erythrocyticsedimentation rate(mm/hr)	05±0.01	05±0.03	10±0.50	06±0.02	07±0.04

Table 7 Effect of AEHS on biochemical parameters

Parameters	Control	Aspirin	AEHS (125 mg/kg)	AEHS (250 mg/kg)	AEHS (500 mg/kg)
SGOT (U/L)	25±0.12	28±0.145	38±0.194	29±0.15	33±0.16
SGPT (U/L)	37±0.18	33±0.17	46±0.234	37±0.19	39±0.2
ALP (IU/L)	67±0.34	77±0.38	147±0.73	80±0.405	88±0.444
Rheumatoid	8±0.03	8±0.04	12±0.06	10±0.05	12±0.06

Factor (IU/ml)					
C-Reactive Protein (mg/L)	4±0.02	4±0.02	24±0.12	4±0.02	6±0.03

3.4. Histopathological studies

Histopathology study shows the differences in the normal ankle joint and formaldehyde induced rat joint. In the EA treated animals, studies show destructive lesions in connective tissue, vascularity into joint space, and granuloma formation. Aspirin treatment showed normal connective tissue with the presence of lesser edema and absence of necrosis.

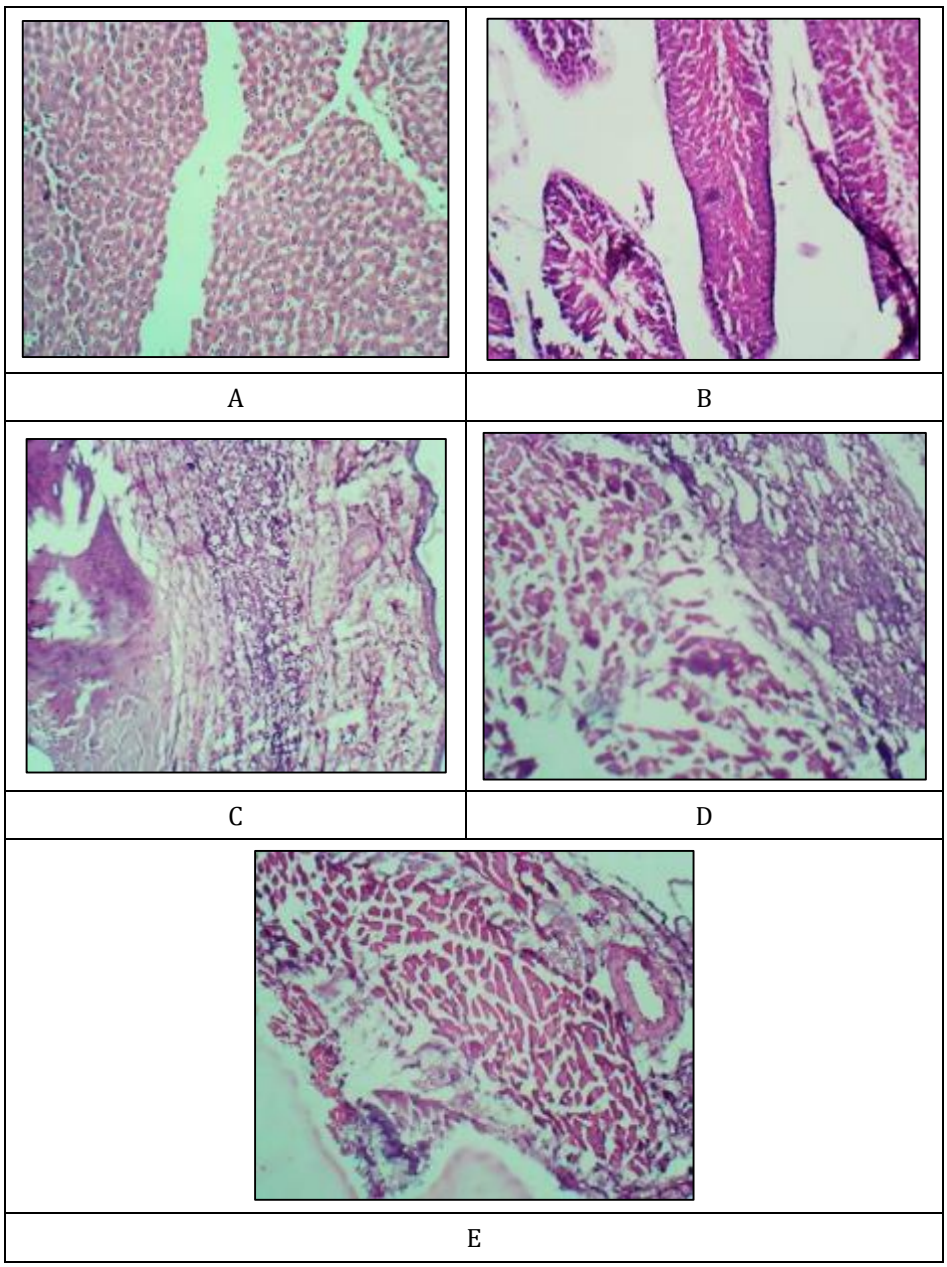

Figure 12 Histology of rat ankle joint - (A) Control; (B) AEHS -125 mg/kg; (C) AEHS – 250 mg/kg; (D) AEHS – 500 mg/kg; (E) Aspirin

By suppressing formaldehyde-induced edema, a common method, anti-proliferative and anti-arthritic efficacy was determined. Formaldehyde injections into animal paws generated inflammation, pain, and histamine, serotonin, and kinin release. Formaldehyde-induced arthritis involves a neurogenic and tissue-mediated phase. The dose-dependent

inhibition of global edematous proliferation by three AEHS dosages was considerable. AEHS (500mg/kg) is stronger than aspirin.

4. Discussion

Inflammatory arthritis preclinical research uses animal models. Formaldehyde-induced arthritis is a well-established rat model used in preclinical research to evaluate anti-arthritic medicines. This study examined the anti-arthritic activity of *Hyptis suaveolens* aqueous extract in vitro and in animals. Phytochemical examination revealed alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenols, terpenoids, proteins, amino acids, glycosides, fixed oils, and lipids. This study reported inhibition of protein (albumin) denaturation, paw and joint edema, and arthritic characteristics.

Protein denaturation destroys tertiary and secondary structure. Extrinsic stress, heat, organic solvent, or strong acid or base destroy protein secondary and tertiary structure. Denaturation involves electrostatic, hydrogen, hydrophobic, and disulphide bonding. In this study, plant extract inhibited protein denaturation as well as diclofenac sodium. The increase in absorbance of test sample compared to control showed that *Hyptis suaveolens* can reduce protein heat denaturation.

Digital plethysmometers monitored formaldehyde-induced arthritic rats' paw volume. The results showed that AEHS 500 mg/kg reduced paw volume as well as Dexamethasone, whereas AEHS 250 mg/kg prevented paw oedema moderately compared to the arthritic control group. Formaldehyde-induced arthritis in rats causes hind paw edema and primary persistent arthritis. In first reaction, prostaglandin generations cause swelling in contralateral and ipsilateral paws (secondary chronic arthritis) and autoantibody production. The right antiarthritic drug should suppress one or both of these phases. Research indicates that proinflammatory mediators such TNF-α, IL-1β, and PDGF have a role in the development of RA.

Patients with persistent arthritis often have anemia. The two most common causes are gastrointestinal blood loss from arthritis drugs and bone marrow alterations in inflammatory arthritis patients that impede iron release for red blood cell synthesis. Arthritic control rats in CFA-induced arthritis had lower RBC, WBC, Hb, and ESR and RF levels. The decline in Hb count during arthritis may be due to lower erythropoietin levels, a slower bone marrow response, and premature red blood cell death. Increased ESR is linked to faster production of endogenous proteins including fibrinogen and α/β globulin, indicating an ongoing but unclear illness process. ESR acute phase proteins increase in concentration in response to stress or inflammation like injection, injury, surgery, and tissue necrosis.

Plasma cells produce IgM antibodies for these complexes. RF measures serum antibody IgM titre. RF is the immune system's response to a nonself immunoglobulin molecule. This reaction to nonself immunoglobulin creates immune complexes that attach to the complement and may damage synovium, cartilage, and bone. More serum RF means more inflammation. To track disease progression and develop new treatments for rheumatoid arthritis, serum RF levels must be measured.

The anti-arthritic activity of a medicine is measured by serum ALP, ALT, AST, and CRP levels. AST and ALT are essential for the synthesis of biologically active chemical mediators such bradykinins in inflammation. Adjuvant-induced arthritic rats may have higher ALP levels due to liver and bone fraction increases. As the enzyme is released into circulation during bone production and resorption, bone erosion and periarticular osteopenia occur. Acute phase proteins like CRP are typical systemic inflammatory biomarkers. The plasma concentration of IL-6, generated by macrophages and adipocytes, rises during inflammation, raising CRP.

Histopathology compares normal ankle joints to formaldehyde-induced arthritic rat joints. Oedema, cartilage degeneration, bone marrow destruction, and widespread infiltration of inflammatory exudates in the articular surface are common histological abnormalities in arthritic joints. Histopathological examinations of hind paw joints in arthritic control rats demonstrated bone marrow loss and significant cell infiltration. By preventing bone from degradation, AEHS(500mg/kg) treatment has significantly reduced all the above clinical conditions, demonstrating its antiarthritic action.

5. Conclusion

This study suggests that *Hyptis suaveolens* seed aqueous extract may be anti-arthritic. It improves haematinic characteristics, clinical indicators such paw edema, joint diameter, and histological investigation during arthritis healing. The impact may be due to aspirin-like phospholipase A2 and prostaglandin inhibition. Aqueous *Hyptis*

suaveolens extract had anti-arthritic effects in this study due to flavonoids, triterpenoid, saponins, tannins, and steroids found following phytochemical screening. The study suggests that *Hyptis suaveolens* supports the traditional usage of the herb to treat inflammatory problems like rheumatoid arthritis. Further research is needed to find and isolate phytoconstituents with anti-arthritic properties to enable the use of *Hyptis suaveolens* in inflammatory disease.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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