1. INRODUCTION

The inflammatory process is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies, or physical injuries)[1]. Although it is adefense mechanism that helps body toprotect itself against infection, burns,toxic chemicals, allergens or other noxiousstimuli, the complex events and mediatorsinvolved in the inflammatory reaction caninduce, maintain or aggravate many diseases [2]. Pain is a subjective experience, hard to define exactly, even though we all know what we mean by it. it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia) or persist long after the precipitating injury has healed (e.g. phantom limb pain). It can also occur as a consequence of brain or nerve injury (e.g. following a stroke or herpes infection [1]. The non-steroidal anti-inflammatory drugs (NSAID) are widely used to treat several inflammatory conditions, however the probability to cause many and severe adverse effects limit their use. In this regard, the traditional medicine continues to use medicinal plants as a substituent to allopathic medicines [3]. The traditional Indian system of medicine has avery long term history of usage in a number of diseases or disorders, but lacks safety and efficacy data for development of standardized safe and effective herbal formulations with proven scientific evidence provide an economical alternative in several diseases areas [7]. A large numbers of Indian medicinal plants are attributedwith various pharmacological activities because they containa diversified class of phytochemicals. It is believed thatcurrent analgesia-inducing drugs such as opioids and nonsteroidalanti-inflammatory drugs are not useful in all cases,because of their side-effects and potency [12].

The present investigation evaluated the anti-inflammatory, analgesic activities of *Kaempferiarotunda* rhizomes to provide experimental evidence for its traditional use. The ethanolic extract (50-55^0 C) prepared from the rhizomes of *Kaempferiarotunda* Linn will be subjected to analgesic, anti-inflammatory studies using animal experiment models which was approved by Institutional and Animal Ethical Committee Phytochemical studies of this extract were also carried out to isolate active constituents.

*Kaempferia*is one of the important genus in Zingiberaceae family which can be found in the Southeast Asia. Plants of the genus are small and herbaceous with short, fleshy or slender rhizomes and one to a few leaves. The *Kaempferia* species can be found in very damp, shaded areas and usually close to streams or in boggy conditions. Several *Kaempferia*species are used to be cultivated in villages for food, spice and folk medicine. Other than that the rhizomes part of *Kaempferia*species has been proven by many researchers to display health benefit properties.

Medicinal plants have been source of widevariety of biologically active compounds for many centuries and used extensively as crude material oras pure compounds for treating various diseaseconditions. The use of herbal medicines becomingpopular due to toxicity and side-effects ofallopathic medicines. Medicinal plants play an important role in the development of potenttherapeutic agents. There are over 1.5 millionpractitioners of traditional medicinal system usingmedicinal plants in preventive, promotional andcurative applications [6]. On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce newmedicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs [2]. Accordingly, anti-inflammatory tests have to be divided into those measuring acute inflammation, sub-acute inflammation and chronic repair processes. In some cases, the screenings directed to test compounds for local application. Analgesia is an ill-defined, unpleasant sensation, usually evoked by an external and internal noxious stimulus. Analgesics are drugs that selectively relieve pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. Analgesics relieve pain, without affecting its cause. Analgesics are divided into two groups, opioid analgesic and non-opioid analgesic. The use of herbal medicines worldwide has provided an excellent opportunity to India to look for therapeutic lead compounds from our ancient system of therapy, i.e. Ayurveda, which can be utilized for development of new drug.This study can open a new phase of treatment of pain and inflammation with the extract of this rhizomes [4].

Inflammation can be classified as either ACUTE or CHRONIC:

Acute inflammationis the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as **chronic inflammation**, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process[14].

- ACUTE INFLAMMATION

Acute inflammation may be due to bacterial pathogens or injured tissues. The cells involved in acute inflammation are neutrophils eosinophils monocytes and macrophages. The primary mediators of acute inflammation are vasoactive amines eicosanoids. The outcomes of acute inflammation are abscesses formation and leads to chronic inflammation. The onset of acute inflammation is rapid and short duration. Bradykinin is responsible for increasing sensitivity to pain. Acute inflammation involves two phase vascular phase and cellular phase. Vascular phase denotes the movement of plasma and antibodies into the inflamed tissue. The inflammatory mediators such as histamine serotonin eicosanoids prostaglandinE_2release nitric oxide and leading to the fluid accumulation in the tissues and cause edema. This fluid contains complement lysozyme which can damage the microbes and help in the preparation of cellular phase. Acute inflammation includes vasodilation causing increased permeability and blood flow causing redness and heat inflammation. Examples of acute inflammation are as follows acute sinusitis, acute dermatitis, acute ingrown toenail etc. [38]

The acute inflammatory reaction is similar whatever the causative agent. The major causes of acute inflammation are:

• Microbial infections- e.g. viruses, pyogenic bacteria

• Hypersensitivity reactions- e.g.Tubercle bacilli, parasites

• Physical agents- e.g. trauma, ionizing irradiation, heat, cold
• Chemicals- e.g. reducing agent, alkali, corrosives, acids, bacterial toxins
• Tissue necrosis- e.g. ischemic infarction.

 Microbial infections - One of the commonest causes of inflammation is microbial infection. Viruses escort to death of individual cells by intracellular multiplication. Bacteria release specific exotoxins (chemicals synthesized by them which specifically initiate inflammation) or endotoxins (which are associated with their cell walls). Additionally, a few organisms cause immunologicallymediated inflammation through hypersensitivity reactions.

 Hypersensitivity reactions – A hypersensitivity reaction occurs when an altered state of immunological reaction causes unsuitable or excessive immune reaction which damages the tissues. The types of reaction are classify as Types I, II, III, & IV, other than all have chemical mediators related to those involved in inflammation.

 Physical agents - Tissue damage leading to inflammation may occur through physical trauma, UV& other ionizing radiation, burns, or excessive cooling ('frostbite').

 Irritant and corrosive chemicals - Corrosive chemicals (acids, alkalis, oxidizing agents) provoke inflammation through gross tissue spoil. Though, infecting agents may liberate specificchemical irritants which produce inflammation.

 Tissue necrosis - Death of tissues from lack of oxygen or nutrients resulting from inadequate blood flow is a potent inflammatory incentive. The boundary of a recent infarct often shows an acute inflammatory response.

 Redness – An acutely inflamed tissue appears red, for example- sunburn, cellulites due to bacterial infection or acute conjunctivitis. This is caused by dilation of tiny blood vessels within the damaged tissues [24].

- CHRONIC INFLAMMATION

Chronic inflammation is due to auto immune reaction non degradable pathogens and persistent acute inflammation. The cells involved in chronic inflammation are monocytes macrophages lymphocytes and plasma cells. The outcomes of chronic inflammation are fibrosis and necrosis and tissue destruction. The onset of chronic inflammation is slow and may be prolonged for many years. Chronic inflammation involves granuloma formation which is a peculiar feature of tubercular and leprosy disease conditions. Examples of chronic inflammation are as follows tuberculosis, leprosy, chronic kidney disease etc. [38]

TYPES OF CHRONIC INFLAMMATION

- NON SPESIFIC

The nonspecific chronic inflammation reaction occurs with the formation of granulation tissue and healing by fibrosis eg: chronic ulcer

- SPECIFIC

Injurious agents produces a characteristic histologic tissue response eg: leprosy

  - It is of two types with the help of histological features
  - Chronic specific inflammation: for eg: chronic oesteomyelitis.
  - Chronic Granulomatous inflammation: leprosy, tuberculosis.

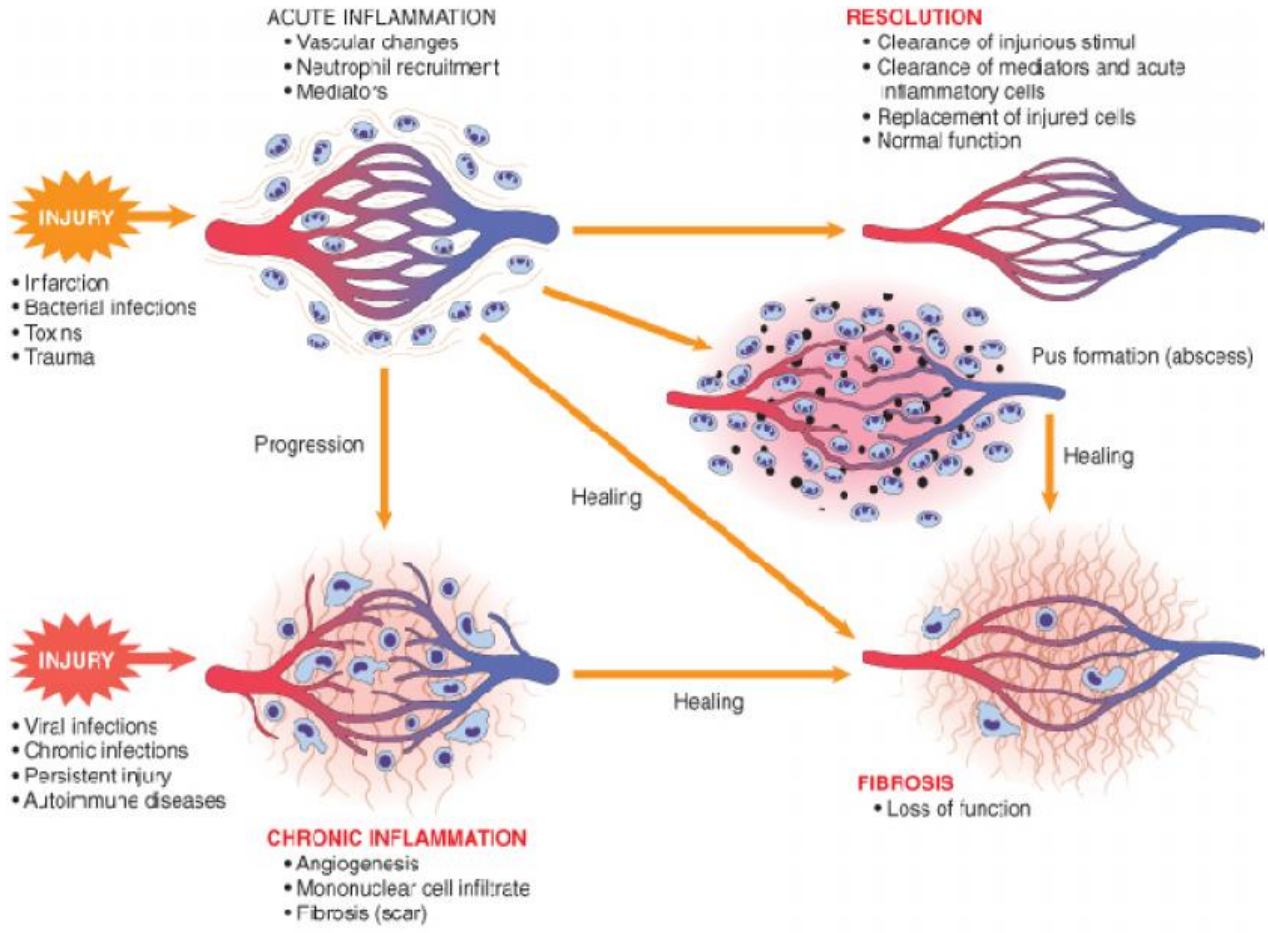

Fig-1: Pathway of Acute and Chronic Inflammation[24]

GRANULOMATOUS INFLAMMATION [39]

Granulomas defined as pool of immune cells mainly histiocytes (macrophages).Granuloma is also known as small module. Macrophages fused with multinucleated giant cells to form epithelial cells. They differ from ordinary macrophages having elongated nuclei which has the appearance of shoe or slipper. Granuloma contain lymphocytes neutrophils fibroblasts. In the infectious and non-infectious disease granuloma formation is seen.Granuloma does not contain a nuclei. The most important feature of delayed hypersensitivity granuloma is the presence of epitheloid cells. certain types of epitheloid cells contain rough surfaced endoplasmic reticulum which is also present in the plasma cells or fibroblasts. The peculiar feature of epitheloid cells is absence of endocytosis material. Granuloma formation is defined as defencemechanism of host from persistent irritants. Granuloma inflammation results in tissue damage.

SIGN OF INFLAMMATION [37]

The cardinal signs of inflammation are:

- ✓ Dolar (pain)
- ✓ Calor (heat)
- ✓ Rubor (redness)
- ✓ Tumor (swelling)
- ✓ Loss of function

AGENT CAUSING INFLAMMATION

- ❖ INFECTIVE AGENTS
  - Bacteria, viruses, fungi, parasites
- ❖ IMMUNOLOGICAL AGENTS
  - Cells mediated
  - Antigen-antibody reactions
- ❖ PHYSICAL AGENTS
  - Heat
  - Cold
  - Radiation
  - Mechanical trauma.
- ❖ CHEMICAL AGENTS
  - Organic and inorganic poisons
- ❖ INERT MATERIALS
  - Foreign bodies

CELL- DERIVED MEDIATORS OF INFLAMMATION

The substances which act as chemical mediators of inflammation may released from the cells, plasma or damaged tissue itself. They are classified into two groups

i. MEDIATORS RELEASED BY CELLS
ii. MEDIATORS ORIGINATING FROM PLASMA

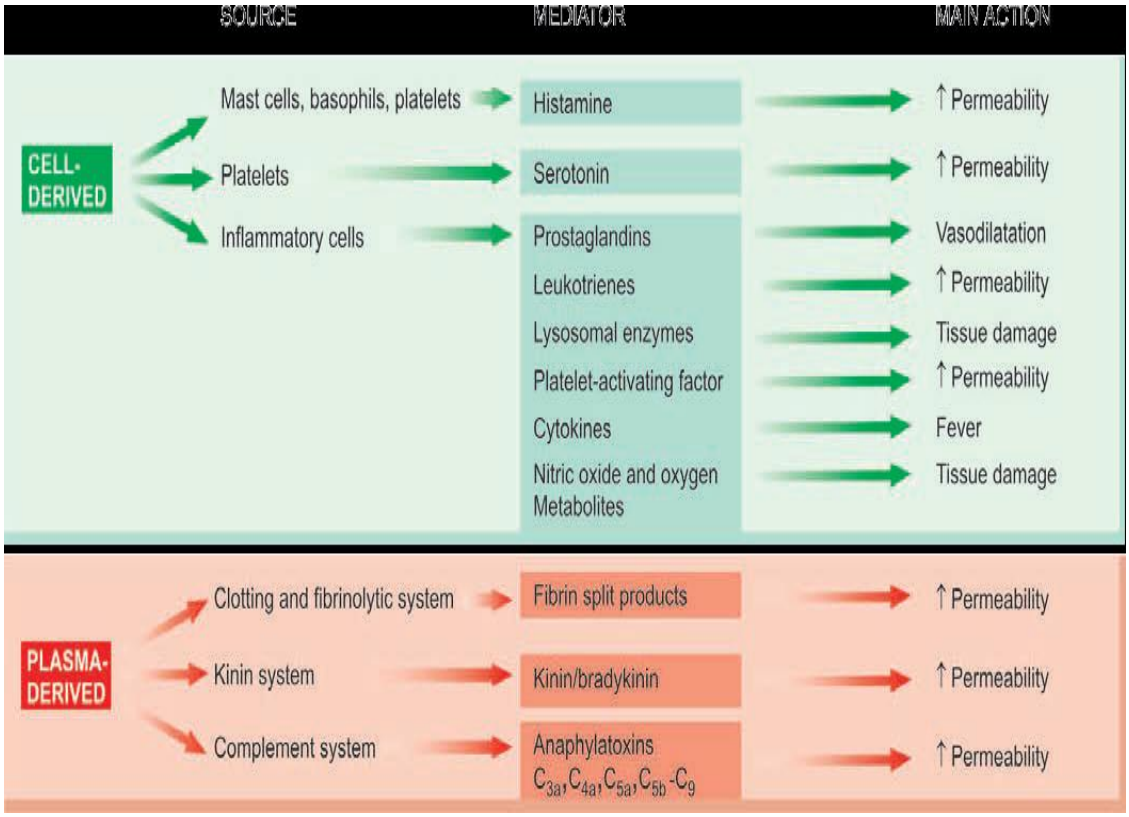

Fig 2: Chemical mediators of inflammation

MECHANISMS OF ACTION OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

Nonsteroidal anti-inflammatory drugs (NSAID) reduce the pain and inflammation by blocking the metabolism of arachidonic acid by cyclooxygenase enzyme (COX), and thereby the production of prostaglandin. There are certain side-effects with use of NSAIDS, like gastrointestinal ulcers, bleeding, and renal disorders, as these drugs nonselectively inhibit both isoforms of COX enzyme. There are also selective COX-2 inhibitors with reduced gastric problems and side-effects but these show adverse cardiovascular effects. Furthermore, the use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects. Therefore, there is a need to developsubstitutes for synthetic drugs and also to search and develop new anti-inflammatory initiatives from natural sources with potent activity and reduced adverse effects [8]. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of fever, acute and chronic arthritic conditions. They act by inhibiting

cyclooxygenase (COX) thereby reducing the release of prostaglandins (PGs), well known inflammatory and nociceptive mediators [18].

Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of the inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of Carrageenan induced inflammation, whereas prostaglandins are detectable in the late phase of inflammation [14].

TREATMENT OF INFLAMMATION

The drugs used for treatment inflammation and the associated pain is by NSAIDS, Opoid analgesic, Steroids, Antihistamines and combined used of analgesic and steroidal drugs. Salicylic acid was prepared by the hydrolysis of bitter glycoside obtained from willow bark. In 1875 sodium salicylate was use as analgesic and antipyretic. This leads to the introduction of acetyl salicylic acid in 1899. Then the next major development was phenylbutazone in 1949. In 1963 indomethacin was introduced.

CLASSIFICATION OF ANALGESIC AND ANTIINFLAMMATORY DRUGS
NONSTEROIDAL ANTIINFLAMMATORY DRUGS

I. Nonselective COX inhibitors

Table-1: Classification of non-selective COX inhibitors

Salicylates	Aspirin
Propionic acid derivatives	Ibuprofen Flubiprofen, Naproxen
Anthranilic acid derivatives	Mephenamic acid
Oxicam derivatives	Tenoxicam, Piroxicam, Meloxicam
Pyrollo pyrrole derivatives	Ketorolac
Indole derivatives	Indomethecin
Pyrazolone derivatives	Oxyphenbutazone, Phenylbutazone

II. Preferential COX_2 Inhibitors
Nimesulide, Nabumetone, Meloxicam

III. Selective COX_2 Inhibitors
Celecoxib, Etoricoxib, Parecoxib

IV. Analgesic Antipyretics with Poor Anti-inflammatory Action
Paracetamol, Metamizol, Nefopam

OPIOID ANALGESICS

I. NATURAL OPIOID ALKALOIDS
Morphine, Codeine

II. SEMISYNTHETIC OPIATES
Pholcodeine, Diacetylmorphine

III. SYNTHETIC OPIATES
Tramadol, Methadone, Dextropropoxyphene, Fentanyl

STEROIDAL ANTIINFLAMMATORY DRUGS

I. NATURAL STEROIDS
Cortisone
Hydrocortisone

II. SYNTHETIC STEROIDS
Dexamethasone
Bethamethasone
Triamcinolone
Prenisolone
Methyl prenisolone
Fludrocortisone

ADVERSE EFFECT OF NSAIDS

- GASTROINTESTINAL

  Gastric irritation, Erosions, Peptic ulcerations, Gastric bleeding, Esophagitis

- RENAL

  Sodium and water retention, Chronic renal failure, Interstitial nephritis, Papillary necrosis (rare)

- HEPATIC

  Increased level of transaminase, Hepatic failure (rare)

- CNS

  Headache, Mental confusion, Behavioural disturbances, Seizure precipitation

- HAEMATOLOGICAL

  Bleeding, Thrombocytopinia, Haemolyticanaemia, Agranulocytosis

- OTHERS

  Asthma, Exacerbation, Nasal polyposis, Skin rashes, Pruritis, Angioedema.

The clinically useful drugs against pain and inflammation exhibitmany adverse effects; this leads to considerable interest in search ofsafer drug for these conditions [30].Pain is an unpleasant sensory and produced by the excitation of particular receptors. Pain can be classified as chronic or acute. The difference between acute & chronic pain is not based on its duration of feeling, other than the nature of the pain itself. Acute pain is symptom of pain. But chronic pain was the "disease of pain''[24]. Acute models are designed to test drugs that modulate erythema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis- polymorphonuclear leucocytes and other phagocytic cells, measurement of local pain, antipyretic activity, local analgesic action and raw paw edema. Chronic models are designed to find drugs that may modulate the disease

process and these include sponge and pellet implants and granuloma pouches which deposit granulation tissue, adjuvant induced arthritis which have an immune etiology [15]. As analgesics, NSAIDs offer the advantage of reducing or avoiding the harmful effects of opioids, but they are not without untoward effects of their own. Drug interactions and untoward effects of NSAIDs are often a direct result of their effect on normal physiologic activity. To understand these effects, it is important to review the normal process of the inflammatory response and the mechanism by which NSAIDs alter it [23].

Need for a new and potent pain therapy

Though the current therapy to the relieve pain are having some limitation such as, Non-steroidal Anti-inflammatory drugs are having GI irritation. Opioids are having the dependency problems. Novel therapies for pain treatment are essential to overcome the adverse effects of existing therapies for pain treatment. It is indeed a need of hour to treat the pain of specific pathologic origin such as cancer pain, neuropathy pain etc. The thirsts for new therapies to treat such painful conditions are alarming. Hence global scenario for new drug discovery is to develop new therapies to treat pain. The study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy, in the search for new
analgesic drugs [30].

MECHANISM OF ACTION OF OPIOIDS

Morphine and other opioids exert their actions by interacting with their specific receptors present on neurons in CNS and in peripheral tissues. Opioid receptors are of three type mu, kappa and delta. Each has a specific pharmacological profile and pattern of anatomical distribution in the brain, spinal cord and peripheral tissues. Opioid receptor are G protein coupled receptors located mostly on pre-junctional neurons. Morphine is a strong analgesic visceral pain is relieved better than sharply defined somatic pain. Peripheral nociceptive fibres activation causes substance p and other pain signal neurotransmitter release from the dorsal horn of spinal cord nerve terminals. Pain signaling neurotransmitter release is regulated by endogenous endorphins or by exogenous opioid agonists to act presynaptically results in inhibition of substance p release causing analgesia. Analgesia is by elevating pain threshold and thus by decreasing the brain awareness of pain.

ADVERSE EFFECTS OF OPIOIDS

- Constipation
- Dry mouth
- Nausea /vomiting
- Sedation
- Sweats
- Hallucination
- Respiratory depression
- Dysphoria
- Urinary retention.

2. LITERATURE REVIEW

2.1 LITERAURE REVIEW ON ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

- **Mathew George et al**[1]evaluated the anti-inflammatory and analgesic activity of its ethanolic leaf extract of*Argyreia nervosa* (family: Convolvulaceae). The phytochemical screening of the ethanolic leaf extract showed the presence of alkaloids, carbohydrate, flavonoids, triterpenoids, proteins, saponins, steroids, tannins, etc. Carrageenam induced paw edema method was used for evaluating potential of plant drug on inflammation and the analgesic activity was evaluated by the acetic acid-induced writhing method and hot plate method. From the study it was evident that the ethanolic leaf extract of Argyreia nervosa possess significant anti-inflammatory and analgesic activity and the study may be helpful to investigate more on the activities of the plant.

- **Ambedkar MohanbabuVittalraoet al**[2]studied the alcoholic extract of *Kaempferiagalanga*was tested for analgesic and anti-inflammatory activities in animal models. Three doses, 300 mg/kg, 600 mg/kg and 1200 mg/kg of the plant extract prepared as a suspension in 2 ml of 2% gum acacia were used. Acute and sub-acute inflammatory activities were studied in rats by carrageenan induced paw edema and cotton pellet induced granuloma models respectively. In both models, the standard drug used was aspirin 100 mg/kg. Two doses 600 mg/kg and 1200 mg/kg of plant extract exhibited significant ($P<0.001$) anti-inflammatory activity in carrageenan model and cotton pellet granuloma model in comparison to control. Analgesic activity was studied in rats using hot plate and tail-flick models. Codeine 5 mg/kg and vehicle served as standard and control respectively. The two doses of plant extract exhibited significant analgesic activity in tail flick model ($P<0.001$) and hot plate model ($P<0.001$) in comparison to control. In conclusion *K. galanga*possesses anti-inflammatory and analgesic activities.

- **Mariana M.G.Pinheiro et al**[3]studied the anti-inflammatory activity of ethanol extract and fractions from *Couroupitaguianensis*aublet leaves and were evaluated in models of inflammatory pain (formalin-induced licking) and acute inflammation (carragenan-induced peritonitis). The ethanol extract, hexane and ethyl acetate fractions of 10, 30 or 100 mg/kg and the reference drugs dexamethasone 5mg/kg, morphine 5mg/kg s.c. and acetyl salicylic acid 100mg/kg p.o. were tested in formalin-induced licking response and carragenan-induced peritonitis. It was found that all three doses from *Couroupitaguianensis*fractions significantly reduced the time that the animal spent licking the formalin-injected paw in first and second phases. However, only higher doses (30and 100mg/kg)wereabletoinhibittheleukocytemigrationintotheperitonealcavityaftercarrageenan injection.Inthis model,the100mg/kg dose almostabolishedthecellmigration.Itwasalsoobservedthat proteinconcentrationresultedfromextravasationtotheperitoneumandnitricoxide(NO)productions weresignificantlyreduced.Cytokinesproductionwasdifferentlyaffectedbythetreatment. TNF-a productionwasreducedafterethanolextractandethylacetatefractionpre-treatment whereashexane fractionhadeffectonlywith100mg/kgdose.IL-1b productionwasinhibitedonlyafterhexanefraction pre-treatment.

- **AvijitChateterjee et al**[4] evaluate the anti-inflammatory and analgesic activity of methanolic extract on medicinal plant *Rhodiolarosea*l. rhizomes. Swiss albino mice are used for acute toxicity study and also analgesic property of the extract by using tail flick, acetic acid induced writhing reflex and tail immersion method. For detecting anti-inflammatory activity male wistar rat were taken using carrageenan induced paw edema. The statistical analysis like one way anova, Dunnet's test was determined. The methanolic extract of *Rhodiolarosea*was administered orally and demonstrate a significant analgesic and anti-inflammatory in animal model.

- **Tej Pratap Singh et al**[5]evaluate the caryophyllene oxide isolated from methanolic extractof bark of *Annona squamosa*and studied its analgesic and anti-inflammatory activity. Caryophyllene oxide were tested at the dose of 12.5 and 25 mg/kg body

weight and methanol extract at the dose of 50 mg/kg wt. showed a significant central as well as peripheral analgesic along with anti-inflammatory activity. it was shown that the activities of caryophyllene oxide were compared with the standard drug Pentazocin (50mg/kg body weight) for anti-inflammatory activities and Aspirin (100 mg/kg body wt.) for analgesic activity used.

- **S. Kumar et al**[6] studied the anti-inflammatory activity of several herbal plants developed the potent anti-inflammatory drugs from the natural products. The studies involved the need for search of newer drugs with less or no side effects. The natural products from medicinal plants plays a major role to cure many diseases associated with inflammation. The present review is directed towards complication of data on promising phytochemicals from the herbal plants that have been tested in inflammation models using modern scientific system.

- **Shyam Kumar and Bhat Ishwar** [7]studied the analgesic,anti-inflammatory activity and phytochemical investigation of *Clitoriaternatea*flower extract. The present study evaluated using petroleum ether (60-80^0 C) extract at the dose of 2000mg/kg body weight by acute toxicity studies. Phytochemical investigation carried out on petroleum ether and the extract reveals the presence of Taraxerol, a pentacylclic triterpenoid which may impart the pharmacological activity of the extract.

- **Pradeep Singh et al** [12]evaluate the anti-inflammatory of ethanolic extract of roots of *Vitex negundo*using carrageenan induced raw paw oedema method for acute inflammation and for cotton pellet granuloma method for chronic inflammation at low dose level (50mg/kg body weight) and high dose level (500 mg/kg body weight). The standard drugs used was Indomethacin (10 mg/kg b.w) in both the models and the result showed that the ethanolic extract at high dose level exhibit remarkable anti-inflammatory activity in both models compared to the standard reference drug indomethacin.

- **Dr. Jagadeesh. K et al**[17]report the anti-inflammatory effect of *Azadirachaindica* (neem) in albino rats. It was carried out for both acute as well as sub-acute

inflammation by using carragenan induced paw edema inhibition and cotton pellet granuloma methods respectively. Ulcer index of Indomethacin and test compound were also studied. The experimental study showed that the neem had significant anti-inflammatory effect in both acute as well as chronic inflammation and also found to have low ulcerogenic potential compared to Indomethacin.

- **Ranjit Thakur et al**[16]studied the antioxidant, antibacterial and anti-inflammatory activity of cinnamon (*Cinamomumtamala*),ginger (*Zingiberofficinale*) and turmeric (*Curcuma longa*) which were determined by measuring FRAP (ferric reducing-antioxidant power) assay for antioxidant activity, paper disc method against different gram negative bacterial and in-vitro anti-inflammatory activity evaluated using proteinase inhibitory assay. Aspirin was used as a standard drug for the study of anti-inflammatory activity. The result shows that the ethanolic extract of the ginger and turmeric were effective against all the bacteria tested, whereas the ethanolic extract of cinnamon was failure in inhibiting the growth of all bacteria tested. The ethanolic extract of ginger possessed strong antioxidant activity in FRAP method. The ethanolic extracts of ginger shows the largest antioxidant FRAP value whereas the turmeric ethanolic extract showed the minimum antioxidant FRAP value.The ethanolic extract of ginger and turmeric also showed in vitro anti-inflammatory activity by inhibiting the proteinase activity. Proteinase activity was significantly inhibited by ginger (78.49%), turmeric (66.48%) and cinnamon (58.72%) at 800ug/ml concentration. From the result it is concluded that the ginger, turmeric and cinnamon ethanol extract showed the antioxidant and anti-inflammatory activity whereas the ginger and turmeric ethanol extract exhibited the antibacterial activity.

- **Bokanisereme et al**[19] studied to evaluate the in-vivo anti-inflammatory, anti-pyretic, analgesic activities and phytochemical analysis of ethanol cassavaleaf extract (ECLE).

  The leaf extract was prepared with ethanol filter and rotary evaporated and stored in desiccator. Preliminary phytochemical screening terpernoids, tannins, flavonoids, anthraquinones, alkaloids, and cardiac glycosides and carotenoids were carried out. A different concentration of the leaf extract was evaluated for their anti-

inflammatory, analgesic and anti-pyretic effect using carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats respectively. The result showed that the extract was able to inhibit carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats. Terpernoids, tannins, flavonoids, carotenoids were found present in the ECLE.

- **Ali Almasiradet al**[21] synthesized analgesic and anti-inflammatory activities of a new methyl-1,3,4-oxadiazoles and 1,2,4-triazoles by molecular hybridization of previously described anti-inflammatory compounds. The target structures were synthesized by preparation 5-methyl-1H-imidazole-4carboxylic acid hydrazide 6 whichwas converted to target compounds 7-15 according to the known procedures The analgesic and anti-inflammatory profile of the synthesized compounds were evaluated bywrithing and carrageenan induced rat paw edema tests respectively. Compounds 8, 9 and 11-13 and 15 were activeanalgesic agents and compounds 8, 9 and 11-13 showed significant anti-inflammatory response in comparisonwith control. Compounds 11 and 13 were screened for their ulcerogenic activities and none of them showedsignificant ulcerogenic activity. The active Compounds 11 and 12 showed the highest drug likeness and drugscore.

- **Rajesh Asija et al**[24]reported the anti-inflammatory and analgesic activity of medicinal plants with various screening models likeCarrageenan induced paw edema, Cotton pellet granuloma method, Formaldehyde-induced paw edema models, Eddy's hot plate method, Formalin test, Acetic acid induced writhingtest, Tail flick method, etc and inducing agents.

- **M. V. Anoop and A. R. Bindu**[25]evaluate in-vitro anti-inflammatory activity of *Syzygiumzeylanicum* leaves by inhibition of Cyclooxygenase, 5-lipoxygenase and by inhibition of protein denaturation. Successive solvent extraction of shade dried leaves were carried out using solvents of increasing polarity and all the extracts were subjected to estimation of total phenolics and the extract containing maximum of phenolics were subjected to anti-inflammatory studies. Preliminary phytochemical analysis was done on all extracts. Ethyl acetate extract and aqueous

extract found to contain maximum of phenolics by Folin-ciocalteau method. Anti-oxidant activity study by using nitric oxide scavenging activity showed the ethyl acetate extract has maximum activity (IC50 = 125 mcg/ ml).The evaluation of anti-inflammatory activity by inhibition of Cyclooxygenase, 5-lipoxygenase and by determination of protein denaturation showed the ethyl acetate extract possess good anti-inflammatory activity.

- **Mahesh S.Kaneria et al**[26]evaluate anti-inflammatory, antiarthritis and analgesic activity of a herbal formulation in animal models. Carrageenin and egg-albumin induced rat hind paw edema performed for acute inflammation, cotton pellet granuloma for chronic inflammation activity and Fruend's adjuvant induced polyarthritis in rats. The product inhibited the increased level of serum lysosomal enzyme activity viz. serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase and the lipid peroxidation in liver. In Fruend's adjuvant induced polyarthritis, herbal product reduced the increased level of hydroxyl proline, hexasamine and total protein content in edematous tissue. The product also exhibited the mild to moderate analgesic activity in acetic acid writhing in mice.

- **Sanmati K. Jain and Pradeep Mishra**[27]explore the analgesic and anti-inflammatory activity of some synthesized 2-substituted acetamido-5-aryl-1,3,4-thiadiazoles. Analgesic activity was determined by using hot wireanalgesiometer. The *in-vivo* anti-inflammatory effects of the thiadiazolecompounds were studied in a carageenan induced rat paw edema model. None ofthe compounds showed any analgesic activity. some compound showed very good anti-inflammatory activity with 51% paw oedema inhibition compared to that of the standard drug and the others with 46% paw oedema inhibition and some moderate anti-inflammatory activity showed 22-37% paw edema inhibition.Results indicate thepotential of these compounds as anti-inflammatory agents which are non-acidic andnon-steroidal.

- **V.VinothPrabhu et al**[30] evaluate anti-inflammatory and analgesic activity of *Tridaxprocumbens*linn against formalin, acetic acid and CFA induced pain models. Phytochemical screening is conducted for both aqueous and ethanolic extracts of the plant using conventional protocol. The analgesic activity is evaluated by two analgesic and one inflammatory in-vivo painmodels male were also studied and it was showed that late phase of moderate pain which starts about 20 min after formalin injection and lasts about 40 min to 60 min, appears to be caused by tissue and functional changes in the dorsal horn of the spinal cord. Administration of extract demonstrated significant inhibition in late phase Similarly, In the acetic acid induced abdominal constriction test, T.P extract dose- dependently and significantly reduced the abdominal writhing. In CFA Induced Hyper analgesia Oral administration of T.P extract significantly reduced mechanical hyper analgesia in CFA injected rats. So, it has been observed that *Tridaxprocumbens*has marked beneficial effects against centrally, peripherally and inflammatory pain models. This protective action may beattributed towards the presence of flavanoid and sterol indicates that the extract of *Tridaxprocumbens*may be used as an effective analgesic.

3. PLANT PROFILE

3.1 LITERATURE REVIEW ON*KAEMPFERIA ROTUNDA*

Scientific Name: *Kaempferia rotunda*

Family: Zingiberaceae

Fig 3: Image of rhizomes and leaves of *Kaempferia rotunda* Linn

Taxonomical Classification

Kingdom:	Plantae
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	Kaempferia
Species:	K. rotunda

Synonym: *Kaempferiabhucampac* Jones

*Zerumbet zeylanica*Garsault

Kaempferia longa Jacq

*Kaempferia versicolor*Salisb.

Vernacular name:

Hindi – bhuichampa

English – black horm, Indian crocus

Kannada – nelasampige

Khasi –ingsmoh

Other – resurrection lily

3.2 BOTANICAL DESCRIPTION

K. rotunda Linn. is a stemless and rhizomatous herb. The leaves are erect, oblong, up to 30cm long, usually variegated with darker and lighter green above along midrib,and tingled purple beneath. The flowers are large, light purple and appear before leaves, from radical spike. Grows best in a moist, well-drained, fertile, humus-richsoil in. The leaves and rhizomes of this species are used to treat sprain and stomachic aches by the locals ofNortheast India [36]. It is found in various parts of India and adjoining regions but seldom in the wild.

3.3 RESEARCH ON THE PLANT

J. PriyaMohanty et al[28]evaluate the anti-oxidant potential of *Kaempferia rotunda* Linn. The anti-oxidant property was assessed by lipid peroxidation markers such as monoaldehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE). The lipid peroxidation byproducts are highly toxic and responsible for various diseases like myocardial infarction, diabetes mellitus, hepatic injury, atherosclerosis, rheumatoid arthritis, and cancer. The chemical constituents of the plant were critically and qualitatively analyzed to confirm the presence of flavonoids and the phenolic derivatives. Hence this helps to evaluate the anti-oxidant effect of *Kaempferia rotunda*linn. and its contribution to control the lipid peroxidation.

S P Geetha et al[31]studied the micro-propagation of*Kaempferia* spp. (*K. galangal L.*and *K. rotunda L.*).The buds produced multiple shoots and well developed roots inMurashige and Skoog medium supplemented with 0.5mgP naphthaleneaceticacid

and 1.0 mgP 6-benzylaminopurine. A multiplicationratio of 1:10 and 1:6 with an average of 7 and 5 roots per shootwas obtained in *K galanga*and *K. rotunda,* respectively. Themicropropagated plants were successfully planted out in pots with over90 per cent survival. The morphological characters and yield of thesetwo species for three crop seasons were also studied.

S. Agarwal et al[33]studied in-vitro anthelmintic activity of *Kaempferia rotunda* Alcoholic extract from the rhizomes of *Kaempferia rotunda* Linn was investigated for their anthelmintic activity against *Pheretimaposthuma*and *Ascardiagalli*. The studies involved the determination of time of paralysis and time of death of worm. The alcoholic extract exhibited significant Anthelmintic activity at highest concentration of Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. The Anthelminticactivity of alcoholic extract of *Kaempferia rotunda* has therefore been demonstrated for the first time.

Syed RashelKabir et al[34] reported the anti-bacterial and anti-proliferative activitiesand isolation, characterization of new lectin [KRL] fromthe tuberous rhizome of *Kaempferia rotunda*. A lectin was purifiedfrom the extract of *Kaempferia rotunda*linn by glucose-sepharose affinity chromatography. KRL was determined to be 29.0± 1.0 kDa polypeptide by SDS-PAGE under both reducing and non-reducing conditions. Methyl-α-D-mannopyroside, D-mannose and methyl-α-D-glucopyranose were the most potent inhibitors. KRL lost its activity markedly in the presence of denaturants and exhibited a high agglutination activity from pH 6.0 to 8.2and the temperature 30-60^{0}C. the lectin showed toxicity against brine shrimp nauplii with the LC_{50} value of 18±6 µg/ml and strong agglutination activity against seven pathogenic bacteria. KRL inhibited the growth of six bacteria partially and did not show antifungal activity. in addition, anti-proliferative activity against Ehrlich ascites carcinoma (EAC) cells showed 51% and 67% inhibition.

Sri Atun et al[35] reported the isolation and antimutagenic activity of some flavanone compounds from *Kaempferia rotunda*. The methanol extract of this rhizomes was

partitionated three times by n-hexane, chloroform,and ethyl acetate respectively. Each fraction was fractionated by vacuum liquid chromatography(VLC) and purified by column chromatography gravitation. Identification structuresof all pure compounds were elucidated based on spectroscopic methods (UV, IR, andNMR) and compared to the spectroscopic previously reported data. Anti-mutagenic activitytest was observed in vivo based on the number of micronucleated polychromatic cellerythrocytes (MNPCE) from male Balb-c mice (8-12 week) induced by cyclophosphamide. The methanol extract and isolated flavanones from K. rotundashowed significant antimutagenic effect compared to control group.

Nur Adilla Che Jamalluddin[36]studied essential oils, phytochemicals and bioactivity of *Curcuma aeruginosa* Aff. and *Kaempferia rotunda* Linn. These oils were analyzed by GC and GCMS.The chemical compositions were identified by comparison of the mass spectraldata of Wiley Library and Kovats Indices with literature values. *K. rotunda* essential oil was found to have33 components with high concentration of benzyl benzoate (31.48%), bornyl acetate(5.56%), camphor (5.45%) and camphene (5.04%). Extractions by soxhlet apparatuswere carried out on the dried samples to get the crude extracts. The structures of theisolated compounds were identified by spectroscopic techniques including IR andNMR (1D and 2D) spectroscopies and mass spectrometry. Fourcompounds were successfully isolated from *K. rotunda* and characterized as benzylbenzoate, crotepoxide, lignoceric acid and stigmasterol. The crude extracts, essentialoils and several pure phytochemicals were screened for antibacterial andantityrosinase activities. Disc diffusion method followed by minimum inhibitionconcentration (MIC) and minimum bactericidal concentration (MBC) against Grampositive and Gram negative bacteria were used for antibacterial assay. All theessential oil, crude extracts and compounds of *C. aeruginosa* and *K. rotunda*exhibited weak to inactive activity against the entire tested microorganisms. Modified dopachrome method with L-DOPA as the substrate was chosen to screenthe antityrosinase activity.Hydrodistillation of the fresh rhizomes of *C. aeruginosa* and *K. rotunda*gave 77.1% and 89.3% oils respectively.

4. AIM & OBJECTIVE

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Pain has an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Many drugs that are currently used for the management of pain are opioids or non-opioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.Asmost of these chemically induced drugs produce some of the side effect.Thus in this study, I have selected the rhizome of *Kaempferia rotunda* as a potent anti-inflammatory and analgesic evaluation and less side effectwhich is commonly used traditional medicine. The rhizome is ground into paste and applied externally for the treatment of sprains for local use. Hence present study was undertaken to evaluate the anti-inflammatory and analgesic activity of *Kaempferia rotunda* in Wistar albino rats using ethanol extract.

PLAN OF WORK

Collection and authentication of plant

Drying of plant under shade

Powdering of rhizome

Extraction of plant material

Phytochemical assay

Acute toxicity studies

Anti-inflammatory activity

(Carrageenan induced paw edema method)

Analgesic activity

(Eddy's hot plate method)

(Tail immersion method)

Results and discussion

Conclusion

5. MATERIALS AND METHODS

5.1 PLANT MATERIALS

The plant was collected from Ri Bhoi district of Meghalaya. The plant was identified by its vernacular name and authenticated by Dr. R. Murugan scientist Botanical Survey of India Coimbatore 641003.

5.2 CHEMICALS AND REAGENT

Mayer reagent, Fehling's A solution, Fehling' B solution, Mercuric chloride, Copper sulphate, Potassium sodium tartarate, Sodium hydroxide, Ferric chloride, Iodine solution, Sulphuric acid, Hydrochloric acid, Mercuric acid, Chloroform, ethanol, carrageenan were used in this study.

5.3 INSTRUMENTS

- Soxhlet Apparatus
- Plethysmometer
- Analgesiometer

5.4 PREPARATION OF EXTRACT

The plant rhizome were collected and cleaned to remove unwanted material. The rhizome were cut into pieces and dried in shed for one week. The dried rhizome of *Kaempferia rotunda* was pulverized to the fine powder and 55gm was extracted with 500ml of ethanol in Soxhlet apparatus. The extract was concentrated by distillation and then the solvent was evaporated to dryness on water bath.

5.5 YIELD AND COLOUR DETERMINATION

The colour of the extract was observed by naked eye. The yield of theextract was determined using the following formula,

% yield = (Weight of the extract/weight of powder taken) x 100

5.6 PRELIMINARY PHYTOCHEMICAL TEST

Small amount of the ethanolic extract of *Kaempferia rotunda* rhizome was investigated to find the presence of different phytochemicals. To determine the presence of phytochemicals standard methods are used

Table- 2: Preliminary Phytochemical Studies of various test

Sl.No	Phytoconstituents	Name of the test	Procedure	Inference
1.	Alkaloids	Mayer's test	1ml of extract+ add 1ml of Mayer's reagent and far drop of iodine solution.	Formation of yellow colour
2.	Terpenoids	_	1ml of extract+ 1ml of conc. H_2SO_4, water bath for 2-4 mins.	Formation of greyish colour
3.	Phenol and tannins	Ferric chloride test	1ml of extract+ 1ml of ferric chloride	Formation of green or black colour
4.	Carbohydrates (sugar)	Fehling's test	1ml of extract+ 1ml of Fehling's A and B solution, water bath for 2-4 mins	Formation of red colour
5.	Saponins	Froth formation test	1ml of extract+ 1ml or 2ml of distill water, shake well	Formation of 1cm foam layer
6.	Flavonoids	Shinoda test	1ml of extract, add few fragment of magnesium ribbon+ few conc.HCL	Appearance of pink scarlet colour

7.	Quinines	_	1ml of extract+ add 1ml of 2% sodium hydroxide	Formation of blue green or red colour
8.	Proteins	Millon's test	1ml of extract, add far drop of mercuric acid or nitric acid	Formation of yellow colour
9.	Steroids	Salkowski test	1ml of extract, add 1ml of chloroform+ 1ml of conc.H_2SO_4siderwise	Red colour produce at the lower chloroform layer

5.7 EXPERIMENTAL ANIMALS

Young adult wistaralbino rats (120- 150g) of either sex used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages under standard environmental conditions (12H dark/ 12H light cycles; temp., 25 ± 2^0 C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum.* The animals were acclimatized to the environment for two weeks prior to experiment use. The experiment was carried out according to the guidelines prescribed by Animal Welfare Board and with the prior approval of animal ethical committee.

5.8 APPROVAL OF PROTOCOL

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of RVS College of Pharmaceutical Sciences, Sulur, Coimbatore constituted under Committee for Purpose of control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 1012/PO/c/CPCSEA). Ethical guidelines were strictly followed during all the experiments.

5.9 TOXICOLOGICAL EVALUATION

Acute toxicity studies

Acute toxicity studies were performed as per OECD-423 guidelines. Swiss albino mice of either sex were divided into three groups with six animals each. Dried rhizome extract of *Kaempferia rotunda* was administered orally to mice at a different dose levels of 50, 200 and 2000mg/kg body weight. The animals were fasted prior to experiment for 18 hrs with free access to water and they were divided into three groups of six animals each. All animals were observed for toxic symptoms and recorded systematically for the first 4 hrs after administration. Further these animals were observed for 24hrs and thereafter once a day for next 14 days. Mortality was recorded during the course of study, if any. The toxicological effect was assessed on the basis of mortality and expressed as LD_{50}.

5.91 PHARMACOLOGICAL EVALUATION

ANTI-INFLAMMATORY STUDIES

Carrageenan-induced Paw Edema

The anti-inflammatory activity of the methanolic extract of *Kaempferia rotunda L.* was assessed by the carrageenan-induced right hind paw edema method.Carrageenan induced paw edema method was used for evaluating potential of plant drug on inflammation. Rats were divided into five groups, six animals each of either sex were used.Plant extracts of 300, 600 and 900mg/kg/p.owere administered orally one hour before the sub plantar injection of 0.1ml of 1% w/v Carrageenan (prepared in CMC stock solution of 100mg in 10ml) in the right hind paw of the rat. Paw volume was measured by plethysmometer immediatelyat 0,1,2,3,4 and 24h intervals after the administration of carrageenan. The control group of animals received vehicle (1ml/kg) orally. Diclofenac sodium at the dose of 10mg/kg body weight was used as a standard reference. The percentage inhibition of edema was calculated for eachgroup with respect to its vehicle-treated control group [2,4]

Percentage inhibition of paw edema = (1–Vt/Vc) × 100

Where Vc represent average increase in paw volume (average inflammation) of thecontrol group of rats at a given time; and Vt was the average inflammation of the drug treated (i.e. plant extracts or test drug diclofenac) rats at the same time.

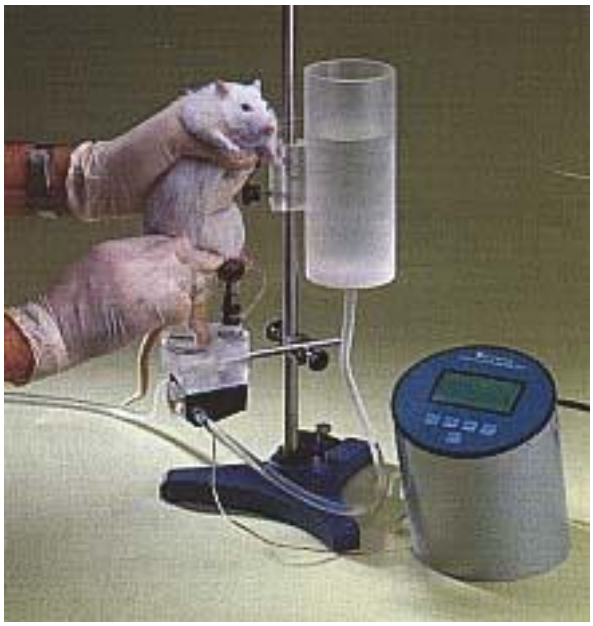

Fig-4: Anti-inflammatory activity on rats using plethysmometer

ANALGESIC ACTIVITY

The central analgesic activity of the test drug was studied against thermal stimuli using the hot plate test and tail immersion method.

Eddy's Hot Plate Method

The hot plate test in mice was performed by Eddy's hot plate method. Animals were individually placed on a hot plate maintained at a constant temperature (55^0C) and the reaction of animals, such as paw licking or jump response was taken at the end point. Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of six mice in each

group for control, positive control and test samples. Each group received a particular treatment i.e. control, positive control (Diclofenac sodium10 mg/kg,p.o) and the test sample (ethanolic extract of 200mg/kg, p.o& 400 mg/kg,p.o respectively).

The animals were positioned on Eddy's hot Plate kept at a temperature of 55±0.5 ^{0}C. A cut off period of 15 s was observed to avoid damage to the paw. The reaction time in control and treated animals was recorded at 0,15, 30, 60 and 90 min after the treatment [13].

Tail Immersion Method

Tail immersion test was used to assess the analgesic activity of *Kaempferia rotunda L.* In this method six rats per group were used. Tail immersion method involved immersing the extreme 3cm of the rat's tail in a water bath containing water at a temperature of (55.0±0.5) °C. After immersing within a few minutes, the rat reacted by withdrawing the tail. The reaction time was noted on a stop-watch. Each animal served as its own control and two readings were obtained for the control at 0 and 10min interval. The average of the two values was the initial reaction time. The test groups were given ethanolic extract of *Kaempferia rotunda L.* (200 mg/kg and 400 mg/kg, p.o.), standard group Diclofenac sodium (10 mg/kg, p.o.) and 1ml/kg saline solution for control group (p.o.). The reaction time of the test groups was taken at 30 min, 1h, 2h and 3h after a latency period of 30 min following the administration of the tests substances. The cut off time, i.e. time of no response was put at 120 seconds. The reaction time was measured and calculated. [4]

6. RESULTS

6.1 YIELD OF ETHANOLIC EXTRACT OF KAEMPFERIA ROTUNDA.

55gm of powdered form of *Kaempferia rotunda* rhizome was extracted with ethanol by using Soxhlet apparatus (60-80^{0}C). The extraction process was continue for 48 hours. Solvent was evaporated to get the solvent free extract. Yield of the extract was found to be 28.45% w/w.

6.2 PRELIMINARY PHYTOCHEMICAL SCREENING.

Qualitative phytochemical analysis test was carried out using several tests as per the standard methods for identification of various constituents. The results of this phytochemical analysis is listed below.

Table 3: **Qualitative phytochemical screening of *Kaempferia rotunda* extract**

PLANT CONSTITUENT	ETHANOLIC EXTRACT OF KAEMPFERIA ROTUNDA LINN.
Alkaloids	+
Terpenoids	+
Phenol and tannins	+
Carbohydrates	–
Saponins	+
Flavonoids	+
Quinines	–
Protein	+
Steroids	+

"+" - Presence

"-" – Absence

6.3 TOXICITY STUDIES

Acute toxicity studies on the albino rats show no morality at a dose of 2000mg/kg, during a time period of 14 days. During the study, no noticeable were seen in the rats. This help to predict that it does not contain any type of toxicity and it is full safe. So 300 mg/kg b.w and 400mg/kg b.w and 900mg/kgb.w were selected of that dose for the further study.

6.4 PHARMACOLOGICAL STUDIES

ANTI-INFLAMMATORY ACTIVITY

The carrageenan induced inflammation was significantly reduced in all the phases of experiment by treatment with 300, 600 and 900mg/kg *Kaempferia rotunda*rhizomes alcoholic extract as well as standard also. But dose 600mg/kg and 900mg/kg of test were found more significant ($p<0.01$) which was comparable to standard drug diclofenac sodium (10mg/kg/p.o) was significant at the level of ($p<0.05$), maximum % inhibition (55.35%) was in 4hr with dose of 600mg/kg of test, (66.30%) with 900mg/kg of the test drug.

Table 4: Anti-inflammatory activity by Carrageenan induced paw edema method

Treatment	Dose mg/kg	Mean paw volume (ml)					
		Initial	1hr	2hr	3hr	4hr	24hr
Control		0.94±0.01	1.81±0.16	1.74±0.06	1.79±0.14	1.68±0.07	0.78±0.04
Diclofenac Sodiums	10mg/kg	0.6±0.09	0.54±0.18 (70.16%)	0.70±0.04 (59.71%)	0.67±0.05 (62.56%)	0.51±0.07 (69.28%)	0.14±0.01 (82.05%)
Ethanolic extract of *K. rotunda*	300mg/kg	0.65±0.06	1.46±0.19 (19.33%)	1.26±0.14 (27.58%)	1.36±0.17 (24.02%)	0.90±0.08 (46.42%)	0.45±0.01 (42.30%)
Ethanolic extract of *K. rotunda*	600mg/kg	0.78±0.08	1.33±0.17 (26.51%)	1.16±0.20 (33.33%)	0.98±0.16 (45.25%)	0.75±0.11 (55.35%)	0.25±0.01 (67.94%)
Ethanolic extract of *K. rotunda*	900mg/kg	0.46±0.07	0.70±0.12 (61.32%)	0.83±0.17 (52.29%)	0.71±0.23 (60.33%)	0.56±0.08 (66.30%)	0.16±0.01 (79.48%)

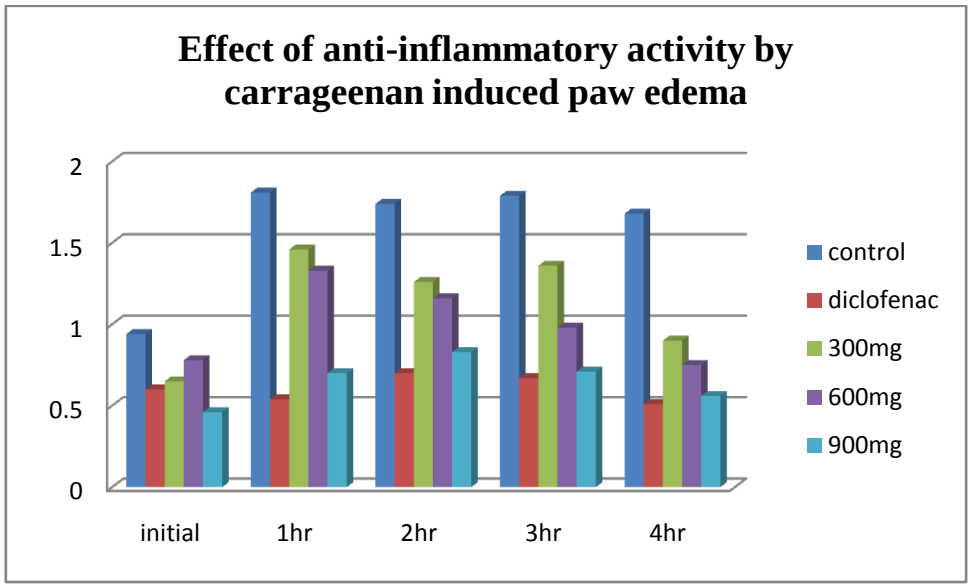

Fig- 5: Effect of anti-inflammatory activity of *K. rotunda* extracts by Carrageenan induced paw edema method

ANALGESIC ACTIVITY

EDDY'S HOT PLATE METHOD (% mean basal reaction time)

The ethanolic extract of Kaempferia rotunda rhizomes (200mg/kg/p.o) did not show significant ($p > 0.05$) increase in the mean basal reaction time in hot plate method compared to control. But extract of dose (400mg/kg/p.o) show very significant ($p < 0.01$) increase in mean basal reaction time when compared with the standard drug diclofenac sodium 10mg/kg/p.o.

Table 5: Effect of analgesic activity of K. rotunda extract by Eddy's hot plate method

Treatment	Dose mg/kg	Reaction time in Sec(Mean±SEM)				
		0 min	15min	30min	60min	90min
Control	10mg/kg	5.16±0.06	5.20±0.05	5.25±0.06	5.31±0.07	5.65±0.07
Diclofenac	10mg/kg	4.85±0.125	7.05±0.12 (35.57%)	8.2±0.10 (56.19%)	10.85±0.12 (104%)	9.41±0.14 (66.72%)
Ethanolic extract of *K. rotunda*	200 mg/kg	2.85±0.22	4.5±0.27 (13.46%)	6.18±0.17 (17.71%)	7.33±0.21 (38.04%)	6.13±0.23 (8.4%)
Ethanolic extract of *K. rotunda*	400 mg/kg	4.21±0.14	6.25±0.18 (20.19%)	7.85±0.19 (49.52%)	9.65±0.07 (81.73%)	8.71±0.22 (54.15%)

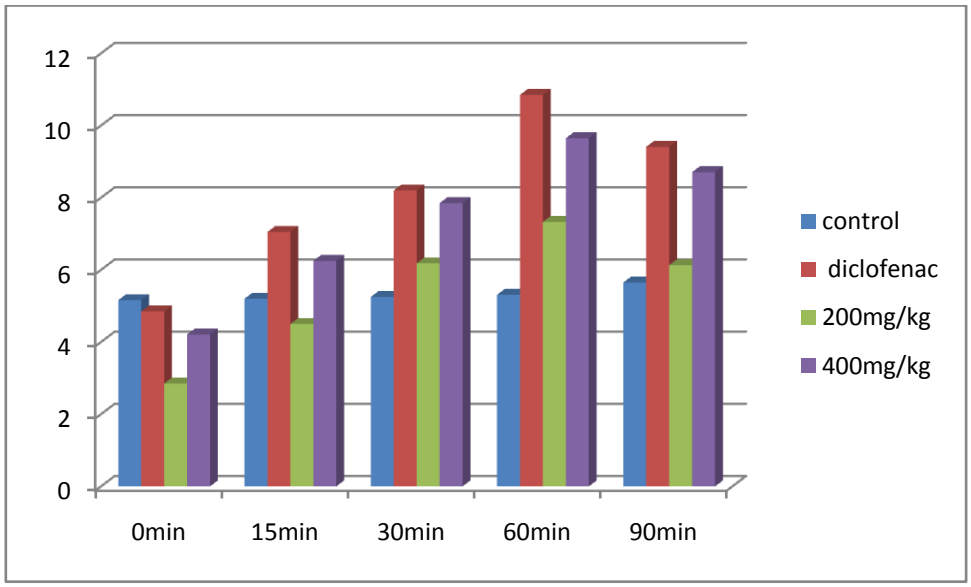

Fig- 6: effect of analgesic activity of K. rotunda ethanolic extract by Eddy's hot plate method

TAIL IMMERSION METHOD

Table- 6: Analgesic activity of *K. rotunda* extracts by tail immersion methods in rats

Treatment	Dose (mg/kg)	0 min	30 min	1hr	2hr	% Inhibition
Control	1ml/kg	3.50±0.18	3.75±0.14	3.83±0.170	3.87±0.11	-
Diclofenac sodium	10mg/kg	3.92±0.22	6.27±0.13	6.72±0.16**	6.65±0.15**	52.87
*K. rotunda*ethanolic extract	200mg/kg	4.35±0.17	4.82±0.10	4.92±0.08	4.57±0.12	5.17
*K. rotunda*ethanolic extract	400mg/kg	3.92±0.23	5.71±0.14	5.90±0.10*	5.88±0.10*	44.59

The data represent the mean±SEM (n=6).ANOVA, Dunnet's test *p<0.05, **p<0.01 compared to corresponding control.

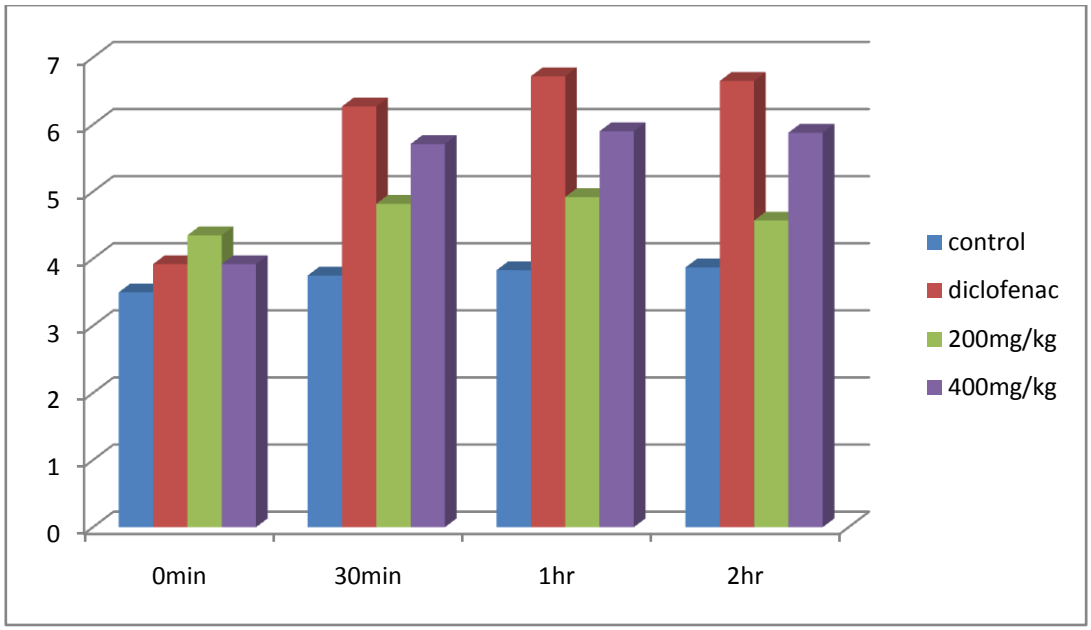

Fig- 7: Effect of analgesic activity of K. rotunda extract by Tail immersion method

7. DISCUSSION

Phytochemical screening

Qualitative Phytochemical screening and ethno botanical survey on K*aempferia rotunda* are evalauted the presence of certain phyto constitutions such alkaloids, flavanoids, protein, steroids,terpenoids, tannins . The phytochemical constituents such as, flavonoids, alkaloids may be linked to the analgesic and anti-inflammatory activity.

Acute toxicity studies

To check the safety profile of the *Kaempferia rotunda*ethanolic extract was subjected to the acute toxicity study which confirmed the absence of any toxicity or mortality at the higher dose of 2000mg/kg. Thus, the ethanolic extract of *Kaemferia rotunda* can be classified as a safe drug category according to the Global harmonized Classification System quoted in the OECD guidelines 1996. Based on the Toxicity studies 50mg/kg is used as a dose of extract and middle dose 200mg/kg and higher dose as 2000mg/kg used for sub-acute toxicity studies.

Anti-inflammatory and analgesic activity

Carrageenan induced paw odema was widely used to screen anti-inflammatory drugs. Screening of anti-inflammatory activity of ethanolic extract of *Kaempferia rotunda* was done by Carrageenan induced paw odema in rat model. While screening of analgesic activity is done by using Eddy's hot plate in swiss albino mice model and Tail immersion method by Wistarrat model. The ethanolic extracts of *Kaempferia rotunda* in varying doses such as (300, 600 and 900mg/kg body weight) was given orally.From the table showed that ethanolic extracts of *Kaempferia rotunda*showed significant reduction in the paw odema at 4hr(600mg/kg 55.35 900mg/kg-66.30) is quite similar when compared to the group treated with standard drug diclofenac sodium (10mg/kg-69.28). Carrageenan induced paw odema in rat model is biphasic in nature.Earlier phase (1-2hr) of the carrageenan model is mainly mediated by histamine, serotonin and increased production of prostaglandins. Then the later phase is sustained by the prostaglandin release mediated by Bradykininleukotrienes, Polymorphonuclear cells . The highest percentage of inhibition was found with the dose of 900mg/kgie, 66.30.

The central analgesic activity of the test drug was studied against thermal stimuli using the hot plate test and tail immersion method.

In case of Eddys hot plate and Tail immersion methods the ethanolic extracts of *Kaempferia rotunda* (200mg/kg,400mg/kg) increase the stress tolerance capacity of animals.In Tail immersion test the time taken to reach the peak analgesic activity +30 and 1hr which is similar to that of standard drug and in Eddys hot plate the time take to reach the peak analgesic activity at +60 minute which is lower to the standard drug +30 minute. From the results it is shown that the ethanolic extracts of *Kaemferia rotunda*possess significant analgesic andanti-inflammatory activity.

8. CONCLUSION

The presented study is an attempt to investigate the Anti-inflammatory and Analgesic activity of ethanolic extract of *Kaempferia rotunda* rhizomes.

The Phytochemical screening showed the presence of Alkaloids, Terpenoids, Steroids, Tannins, saponins, flavonoids and proteins which is responsible for Analgesic and Anti-inflammatory activity.

The finding of the preseninvestigation suggests the Ethanolic extract of*Kaempferia rotunda* has potential for its evaluation as an Analgesic and Anti-inflammatory activity.

Toxicity studies do not produce any toxic symptoms on Acute toxicity study.

In conclusion the Ethanolic extract of *Kaempferia rotunda* are safe and can be used as Analgesic and Anti-inflammatory without any harmful effects.

Further studies are required to confirm the exact mechanism behind the Analgesic and Anti-inflammatory activity of *Kaempferia rotunda*ethanolic rhizomes extract.

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