ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY SCREENING OF ETHANOLIC EXTRACT OF *CHROMOLAENA ODORATA* ROOT

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The Tamil Nadu Dr.M.G.R Medical University Chennai

In partial fulfillment of the requirements for the award of the degree of
MASTER OF PHARMACY
IN
BRANCH – IV – PHARMACOLOGY

Submitted by

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CERTIFICATES

CERTIFICATE

This is to certify that this dissertation entitled **"ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY SCREENING OF ETHANOLIC EXTRACT OF *CHROMOLAENA ODORATA* ROOT"** submitted by **KARTHIKEYAN R** (261825652) at the **Department of Pharmacology, R.V.S COLLEGE OF PHARMACEUTICAL SCIENCES, SULUR, COIMBATORE- 641402** for the partial fulfillment of the university rules and regulations for the award of **MASTER OF PHARMACY IN PHARMACOLOGY,** under my guidance and supervision during the academic year 2018-2020.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY SCREENING OF ETHANOLIC EXTRACT OF *CHROMOLAENA ODORATA* ROOT"** submitted **by, KARTHIKEYAN R** bearing Reg.No**.261825652** to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment for the Degree of **MASTER OF PHARMACY IN PHARMACOLOGY** is a bonafied work carried out during the academic year 2018-2020 by the candidate at Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore and was evaluated by us.

Internal Examiner **External Examiner**

Dedicated to my parents,teachers and friends

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SL.NO	ABRIVATIONS	EXPLANATIONS
1	OECD	Organization For Economic Co-Operation And Development
2	CPCSEA	Committee For The Purpose Of Control And Supervision Of Experiment On Animal
3	IAE	Institutional animal ethics commette
4	CTRI	Clinical Trial Registry Of India
5	ml	Milli Litter
6	2N	2 Normal
7	g, gm	Gram
8	^{0}C	Degree Celsius
9	mg	Milli Gram
10	mm	Milli Meter
11	ng	Nano Gram
12	dl	Desi Litter
13	cm	Centi Meter
14	kg	Kilo Gram
15	hr	Hour
16	µg	Micro Gram
17	CMC	Carboxy Methyl Cellulose
18	ECD	
19	Ppb	Parts Per Billion
20	PHF	Poly Herbal Formulation
21	H_2SO_4	Sulphuric Acid
22	HNO_3	Nitric Acid
23	CNS	Central Nervous System
24	ANS	Autonomic Nervous System
25	LD_{50}	Lethal Dose
26	ED_{50}	Effective Dose
27	NMT	Not More Than
28	SPSS	Software For The Purpose Of Social Service
29	ANOVA	Analysis Of Varience
30	STZ	Streptozotocin
31	i.p	Intra Peritoneal
32	OGTT	Oral Glucose Tolerance Test
33	GL	Glibenclamide
34	%IG	Percentage Induced Glycaemia
35	W/V	Weight By Volume
36	%w/w	Percentage Weight By Weight.
37	SEM	Standard Error Mean
38	RBC	Red Blood Cells
39	WBC	White Blood Cells
40	MCV	Mean Corpuscular Volume
41	MCH	Mean Corpuscular Haemoglobin

42	MCHC	Mean Corpuscular Haemoglobin Concentration
43	Hb	Haemoglobin
44	N	Neutrophils
45	E	Esinophils
46	B	Basophils
47	L	Lymphosites
48	M	Monosites
49	ALT	Alanine Transaminase
50	AST	Aspartate Transaminase
51	SGOT	Serum Glutamate Oxalate Transaminase
52	SGPT	Serum Glutamate Pyruvate Transaminase
53	HDL	High Density Lipoprotein
54	LDL	Low Density Lipoprotein
55	VLDL	Very Low Density Lipoprotein
56	SGL	Serum Glucose Level
57	MTD	Maximum Tolerable Dose

INTRODUCTION

INTRODUCTION

An inflammatory response appears after activation of a immune response. Inflammation is a response to remove the primary cause of cell injury, dead cells, damaged tissues and to initiate the repair process. Inflammation may be of two types acute and chronic. Acute inflammation is the primary response towards a harmful stimulus and is developed by the body due to increased movement of leukocytes plasma from the blood into the damaged cells and tissues. Acute inflammation may be prolonged into chronic inflammation which leads to change in nature of the cells at the inflammatory site. The interaction between microbial invasion and inflammatory response of the body is described as infection. People suffer from inflammation; they feel pain discomfort and stiffness. Pain may be constant and steady. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential damage. Pain may be acute or chronic. Nociceptive pain is due to stimulation of peripheral nerve fibres. Rheumatoid arthritis and other inflammatory disease conditions has become the major health concerns of the world, especially to the geriatric community. Most of the NSAIDS and other steroidal drugs used for long time may lead to health problems such as gastric bleeding ulcers kidney damage and these side effects may be minimal with herbal medicines[1].

AGENTS CAUSING INFLAMMATION[2]

1. INFECTIVE AGENTS
   Bacteria, viruses, fungi , parasites

2 IMMUNOLOGICAL AGENTS
   Cell mediated
   Antigen-antibody reactions.

3 PHYSICAL AGENTS

   Heat, Cold, Radiation, Mechanical trauma.

4 CHEMICAL AGENTS

   Organic and Inorganic poisons

5 INERT MATERIALS

   Foreign bodies

SIGNS OF INFLAMMATION[3,4]

The cardinal signs of inflammation are

- Dolor(pain)
- Calor(Heat)
- Rubor(Redness)
- Tumor(Swelling)
- Loss of function

Aulus Cornelius ceisus [5]describes the first four signs of inflammation while loss of function is described by Galen[6].

TYPES OF INFLAMMATION

Inflammation can be classified as

- ACUTE INFLAMMATION
- CHRONIC INFLAMMATION

ACUTE INFLAMMATION

Acute inflammation may be due to bacterial pathogens or injured tissues. The cells involved in acute inflammation are neutrophils, eosinophil, monocytes and macrophages. The primary mediators of acute inflammation are vasoactive amines eicosanoids. The outcomes of acute inflammation are abscess formation and leads to chronic inflammation. The onset of acute inflammation is rapid and is of short duration. Initiation of acute inflammation is by immune cells such as macrophages kupffer cells mastocytes present in inflamed tissues. During an infection injury or burns these cells are activated and release inflammatory mediators responsible for the cardinal signs of inflammation. 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 prostaglandin E2 release nitric oxide and leading to fluid accumulation in the tissues and causes edema. This fluid contains complement lysozyme which can damage the microbes and helps in the preparation of cellular phase. Acute inflammation includes vasodilation causing increased permeability and blood flow causing redness and heat of inflammation. Examples of acute inflammation are as follows

Acute sinusitis, Acute dermatitis, Acute ingrown toenail etc

CHRONIC INFLAMMATION

Chronic inflammation is due to auto immune reactions non degradable pathogens and persistant acute inflammation. The cells involved in chronic inflammation are monocytes macrophages lymphocytes and plasma cells. The primary mediators chronic inflammation are cytokines hydrolytic enzymes. 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.

TYPES OF CHRONIC INFLAMMATION

- NON SPECIFIC

  The nonspecific chronic inflammatory 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 osteomyelitis.
- CHRONIC GRANULOMATOUS INFLAMMATION : leprosy, tuberculosis

GRANULOMATOUS INFLAMMATION[12]

Granuloma is defined as a pool of immune cells mainly histocytes (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 have the appearance of a shoe or slipper. Grauloma contains lymphocytes, neutrophils, fibroblasts. In infectious and non infectious disease, granuloma formation is seen. Grauloma does not contain a nuclei. Granuloma may be round or oval lesion and is of 1 mm in diameter. The most important

feature of delayed hypersensitivity granuloma is the presence of epithelioid cells. Certain types of epithelioid cells contain rough surfaced endoplasmic reticulum which is also present in plasma cells or fibroblasts. The peculiar feature of epithelioid cells is the absence of endocytosed material. Granuloma formation is defined as a defence mechanism of the host from persistant irritants. Granuloma inflammation results in tissue damage.

CELLULAR EVENTS IN INFLAMMATION

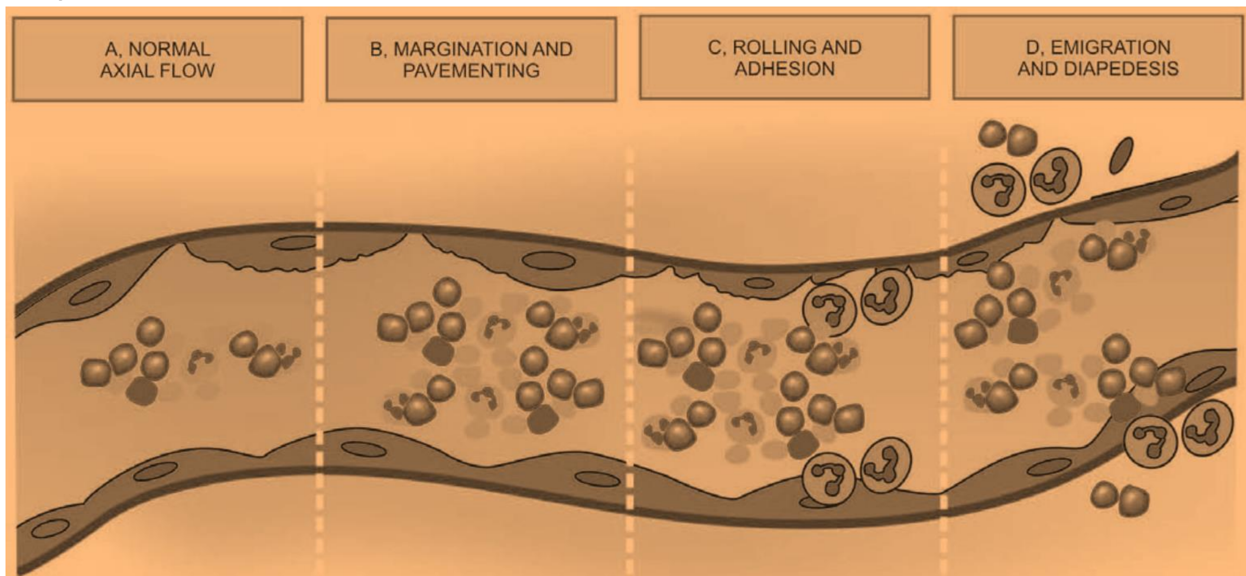
LEUCOCYTES EXUDATION

PHAGOCYTOSIS

LEUCOCYTES EXUDATION: from the lumen of micro vasculature to the interstitial tissues is the most important feature of inflammaton variations due to migration of leucocytes are

- CHANGES IN THE FORMED ELEMENTS OF BLOOD
- ROLLING AND ADHESION
- EMIGRATION
- CHEMOTAXIS

Fig -1 Cellular Events In Inflammation



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CHANGES IN THE FORMED ELEMENTS OF BLOOD

The flow rate of blood is increased in the early stage of inflammation due to vasodilation followed by slowing or stasis of blood stream . Microcirculation takes place due to the decreased flow of blood . The normal flow involves cells consists of leucocytes and RBC's. plasma is closed to the vessel wall. The central stream of cell widens due to stasis of blood flow and the peripheral plasma area becomes shorter due to plasma loss by exudation.

ROLLING AND ADHESION

The pavemented and marginated neutrophils roll over the endothelial cells lining of the vessel wall slowly. Next step is a bond formation between the leucocytes and endothelial cells.

EMIGRATION

After sticking of neutrophils to endothelium, the neutrophils move with the endothelial surface till a suitable site between the endothelial cells is formed where the neutrophils throw out cytoplasmic pseudopods. The neutrophils then filled between the basement membrane and endothelial cells. During the first 24 hours the dominant cells of acute inflammatory exudates are neutrophils and the monocyte & macrophages appear in the next 24-48 hours. Microcyte macrophages survive longer. .Between the endothelial cell gaps the emigration of leucocytes occurs.

CHEMOTAXIS

The transmigration of leucocytes to reach interstitial tissues with the help of chemotactic factor is known as chemotaxis. Chemotaxis is of two types namely positive chemotaxis and negative chemotaxis. The movement towards a higher concentration is called positive chemotaxis while movement towards a lower concentration is called negative chemotaxis. Agents act as chemotactic substances or chemokines are

Leukotriene (LT-B4) : A product of lipogenase pathway of arachidonic acid metabolites . C5a and C3a.

Cytokines: Soluble products of bacteria

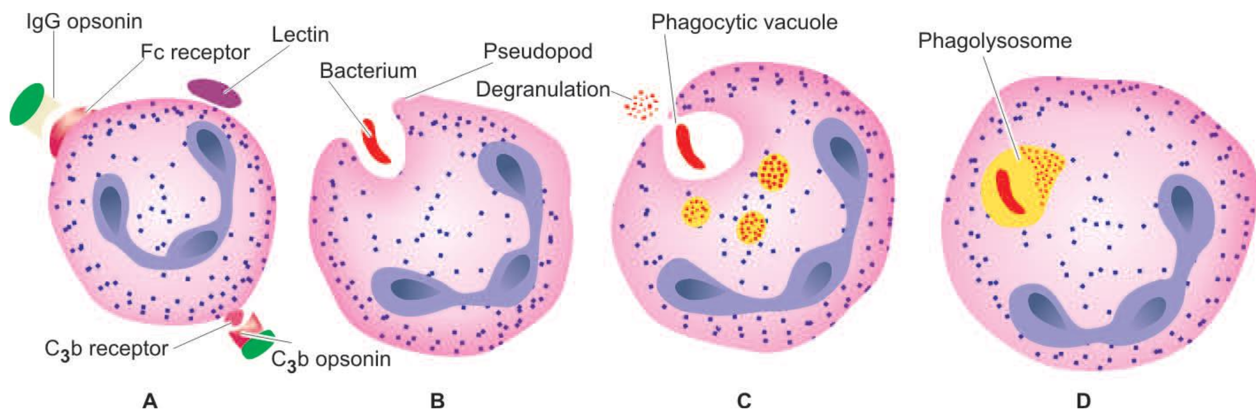
PHAGOCYTOSIS

Phagocytosis is defined as engulfment of solid particle which result in the formation of vesicle internally called phagosome. William Olser[7] noted phagocytosis first and it is later studied by Elie Metchnikoff. The function of phagocytosis is carried out by phagocytes. Phagocytic cells are of two types

- PMNs (Polymorphonuclear neutrophils) or macrophages appears early in acute inflammatory response .
- Fixed tissue mononuclear phagocytes and circulating monocytes and, commonly called as macrophages.

Macrophages and neutrophils synthesize various proteolytic enzymes when they reach the tissue space. Various proteolytic enzymes are acid hydrolases protease lipase and gelatinase. Macrophages and polymorphs involves in the process of engulfing microbes this process is carried out by

Fig -2 Various steps in phagoctosis[2]



A Identification of invaded particle.

B The invaded particle engulfed by pseudopod

C Incorporation within the cell (phagocytic vacuole) and degranulation.

D Formation of phagolysosome

1. Recognition and attachment
2. Engulfment
3. Killing and degradation

1. RECOGNITION AND ATTACHMENT

A bond is formed between cell membrane of phagocytic vesicle and bacteria by opsonins. Initiation of phagocytosis is by macrophages when the surface receptor of macrophages identifies the microorganisms. Phagocytosis process is increased when opsonins specific proteins from the serum coated the microorganism.
The antibody naturally occurring in the serum ie, IgG opsonin is responsible for coating bacteria.

2. ENGULFMENT

The invaded particle attaches to phagocytic surface and is engulfed resulting in the formation of cytoplasmic pseudopods by the activation of actin filaments below the cell wall. The opsonised particle is covered by a phagocytic vacuole and the plasma membrane surrounding the particle breaks and the membrane lined phagosome lies inside and free in the cell cytoplasm. Phagolysosome , the bigger vacuole is formed when the phagosome combines with one or more lysosomes.

3. KILLING AND DEGRADATION

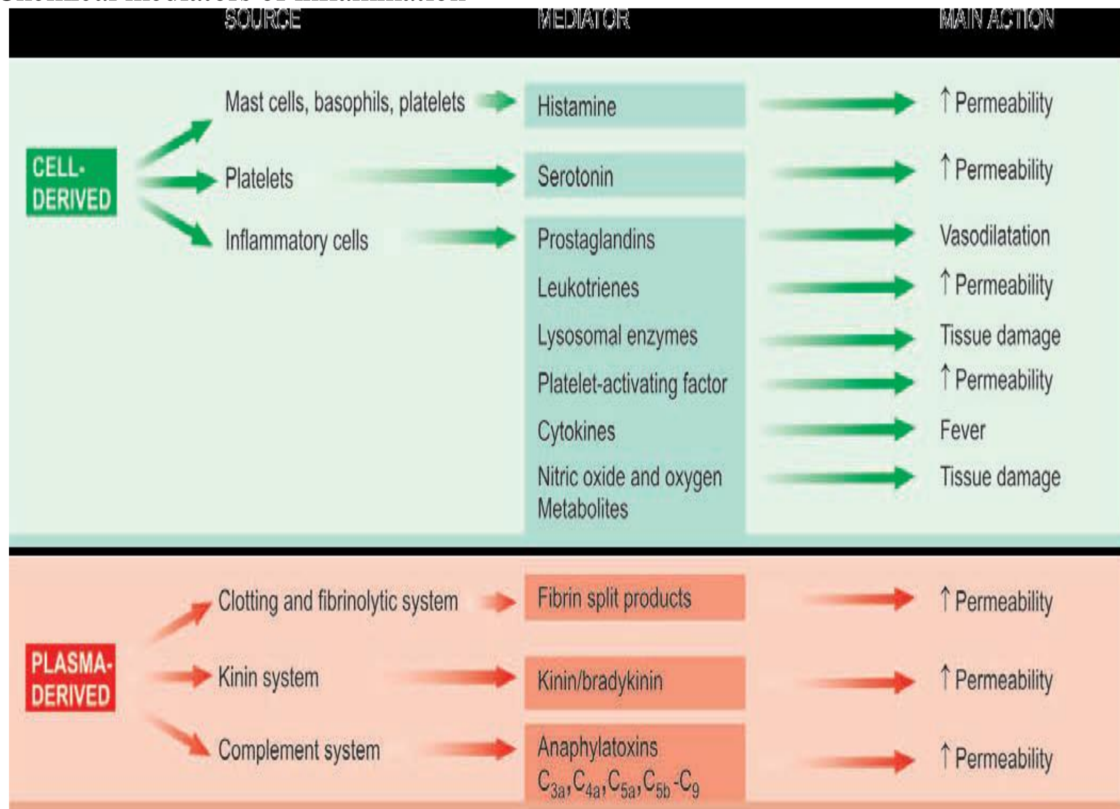
Degradation and killing of microorganism is the next step to dispose it off justifying the function of phagocytes as scavanger cells. The microorganisms after being killed by antibacterial substances are degraded by hydrolytic enzymes. Bacteria like tubercle bacilli cannot be killed and degraded by this process.

CELL-DERIVED MEDIATORS OF INFLAMATION

The substances which act as chemical mediators of inflammation may be released from the cells, plasma or damaged tissue itself. They are classified into 2 groups

- MEDIATORS RELEASED BY CELLS
- MEDIATORS ORGINATING FROM PLASMA

Fig -3 Chemical mediators of inflammation [2]



CELL DERIVED MEDIATORS

VASOACTIVE AMINES

Two vasoactive amines play pivotal role in early inflammatory response are histamine and 5-hydroxy tryptamine(5HT) or serotonin.

- HISTAMINE

  Histamine act as a neurotransmitter and maintains the pharmacological function of the gut[8]. Histamine is involved in immune responses. It is a nitrogenous organic compound. There are two tautomeric forms of histamine. Histamine is found in connective tissues brain. Basophils and mast cells produce histamine and is generated in mast cell granules basophils eosinophils and enterochromaffin cells present in stomach. When allergens bind to IgE sensitized Bsophils and Mast cells they trigger the release of histamine which is immunologic. Drugs like morphine polymixin antibiotic curare alkaloid triggers histamine release. Histamine mainly exert their pharmacological action by binding to histamine receptors mainly h1. The histamine receptors are G-protein coupled receptors.

Histamine has other important role in the body such as gastric acid release, erection, schizophrenia[9]. Vasodilatation, increased vascular permeability, itching and pain are the most important actions mediated by histamine. Mast cells and basophils stimulation release the products of arachidonic acid metabolism and also slowreacting substances of anaphylaxis *(*SRS-As*)* includes various leukotrienes (LTC4, LTD4 and LTE4).

- **5-**Hydroxytryptamine (5-HT or serotonin).

Serotonin is derived biochemically from an aminoacid tryptophan. Serotonin is present in central nervous system ,gastrointestinal tract, bloodvessels. Serotonin act as a neurotransmitter[(10.11)]. The majority of serotonin ie, nearly about 90% is located in Enterochromaffin cells located in G.I.T responsible for intestinal movement. Sleep mood and appetite is regulated by serotonin. The cognitive factors such as learning and memory are also maintained by serotonin. Serotonin is stored in blood platelets. Platelets when binds to a clot release serotonin which act as a vasoconstrictor.

Neuropeptides.

Another class of vasoactive amines is tachykinin neuropeptides, such as substance P, neurokininA, vasoactive intestinal polypeptide (VIP) and somatostatin.

The major pro-inflammatory actions of these neuropeptides is as follows

a) Increased vascular permeability.

b) Transmission of pain stimuli.

c) Mast cell degranulation.

- ARACHIDONIC ACID METABOLITES (EICOSANOIDS).

Eicosanoids also known as Arachidonic acid metabolites and they are most important mediators of inflammation. Phospholipases release arachidonic acid from cell membrane Arachidonic acid is presentin liver brain and muscles[13]. The arachidonic acid is activated by either of the following pathways

Cyclo oxygenase or lipooxygenase pathway

Metabolites via cyclo-oxygenase pathway[14]:

Metabolites of cyclo oxygenase pathway include prostaglandins prostacyclin and thromboxane A_2 . Autacoids are local hormones and they have a pacrine effect and is of short duration. Autacoids are of two types mainly vasoconstrictor and vasodilator.

Prostaglandins and their associated compound is also known as autacoids. Arachidonic acid is activated to prostaglandin endoperoxidase(PGG2) by cyclo oxygenase enzymes COX-1 and COX-2 . PGG2 transferred into PGH2 by enzymatic reaction with the release of free radical of oxygen. It is enzymatically converted into the following metabolites.

Prostaglandins (PGD2, PGE2 and PGF2-α)[15]

Bronchoconstriction and vasodilatationis induced by PGF2-α.Vasodilatation and bronchodilatation is mediated by PGD2 and PGE2 by acting on blood vessels.Prostaglandins causes brain masculinisation in rats.

Thromboxane A2 (TXA2)

Vasoconstrictor and broncho-constriction is mediated by thromboxane A2.The enzyme thromboxane synthase is present in platelets.

Prostacyclin (PGI2).

Bronchodilatation, vasodilatation and platelet aggregation are induced by PGI2 .

Resolvins[16]

Resolvins are the new derivative of COX pathway. Resolvins are helpful to many drugs such as aspirin act by inhibiting COX activity. The anti-inflammatory activity of drugs is mediated by blocking the action of enzyme.

COX; e.g. non-steroidal anti-inflammatory drugs (NSAIDs),

COX-2 inhibitors.

MEATABOLITES VIA LIPO OXYGENASE PATHWAY

They are 5HETE, Lipoxins and Leukotrienes

5HETE – potent chemotactic agent for neutrophils.

Leukotrienes[17] – They are isolated from leukocytes. Smooth muscle contraction vasoconstriction and bronchoconstriction , inflammation in allergic rhinitis and asthma is mediated by LTD4 LTE4 and LTC4[18].

Lipoxins- They are the latest product of lipo oxygenase pathway.

Lysosomal components – mainly granules of neutrophils

Granules of neutrophils involves in primary secondary and tertiary granules.

Primary granules contain active enzymes such as acid phosphatase,phospholipid etc. Secondary granules contains vit B12 plasminogen activator and gelatinase. Tertiary granules contain acid hydrolases and gelatinase enzymes.

- PLATELET ACTIVATING FACTOR (PAF)[19]

Platelet aggregation inflammation and anaphylaxis is mediated by platelet activating factor. PAF helps in communicating between adjacent cells. Allergic reaction is initiated by PAF[20]. PAF levels are elevated in certain conditions like stroke, Allergic reactions, Myocardial infarction, Colits etc[21].

- CYTOKINES[22]

The cytokines involved in the inflammation are IL-1, TNF-α, TNF-β. Oxidative stress induced cytokine levels. Elevated levels of cytokinine level gives about the presence of inflammatory process in autoimmune disease like arthritis and also in plasma of HIV infected patients[23].

- FREE RADICALS

Oxygen metabolites and Nitric oxide act as a potent mediators of inflammation

Oxygen metabolites – Activated macrophages and neutrophils release oxygen metabolites.

Nitric oxide- Nitric oxide is formed during oxidation of arginine by endothelial cells.
Inflammation vasodilation is mediated by nitric oxide.

PLASMA DERIVED MEDIATORS[24]

These include complement system, Fibrinolytic system, Clotting system and Kinin system. Hageman factor plays an important role in connection with the four systems. Permeability factor is the endpoint of the above systems. The permeability factor is responsible for active ation of clotting system

- KININ SYSTEM[25]

This is activated by X11a factor which is responsible for bradykinin release. By the action of prekallikrein activator a factor of X11a on plasma prekallikrein thus kallikrein is formed. Bradykinin is formed when kallikrein acts on kininogen.

- CLOTTING SYSTEM

Fibrinogen is formed by a cascade of clotting system activate by factor X11a. Fibrin and Fibrinopeptides are formed by the action of fibrinogen with thrombin. Anticoagulant activity Chemotaxis are the main actions of Fibrinopeptides.

- FIBRINOLYTIC SYSTEM

Plasminogen activator is responsible for the activation of this system. Plasmin is formed by the action of plasminogen activator with plasminogen a component of plasma proteins. Fibrinopeptides are formed by the breakdown of fibrin by plasmin.

The main functions of plasmin are

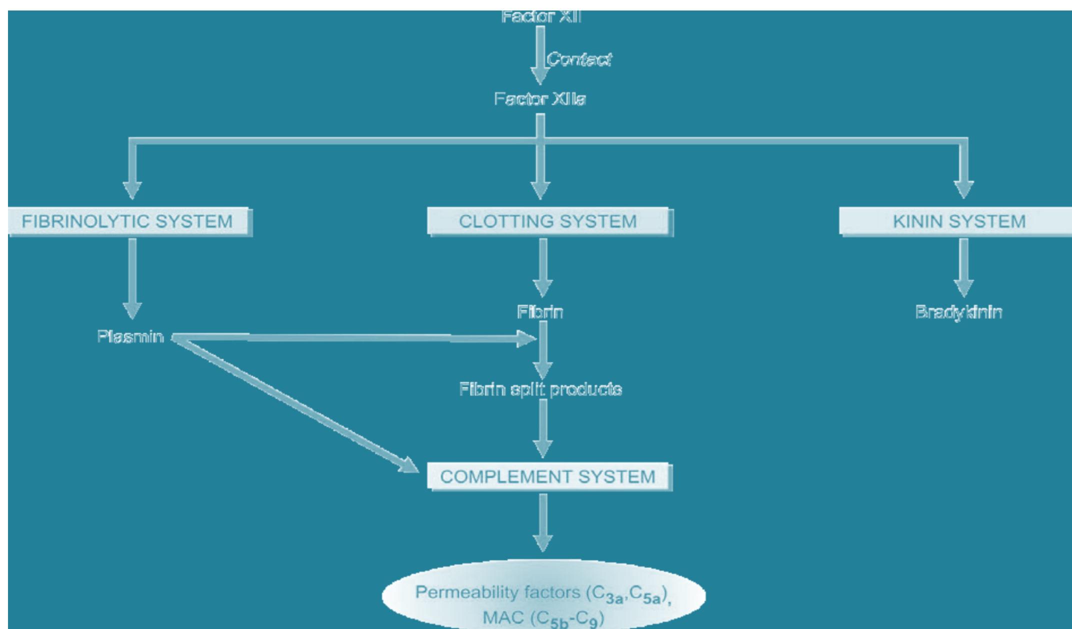
Generation of bradykinin

Splitting of C3-C3a

- COMPLEMENT SYSTEM[26]

It is activated either by antigen antibodycomplex pathway or by non immunologic agents. The products of complement system are C3a C4a C5a (anaphylatoxins) and C5b C6b (MAC).

Fig -4 Complement System.[2]



THE INFLAMMATORY CELLS PARICIPATING IN ACUTE AND CHRONIC INFLAMMATION

In acute and chronic inflammation the participating inflammatory cells are circulating leucocytes plasma cells and tissue macrophages..

- POLYMORPHO NEUTROPHILS (PMNs)

They are called neutrophils or polymorphs. These cells together with basophils and eosinophils are known as granulocytes. They are called so because they contain granules in their cytoplasm[27]. Granules contain substances like myeloperoxidase lysozyme and alkaline phosphatase. Neutrophils have a diameter ranges from 10 to 15 µm and are motile. Neutrophil count is increased in acute blood and tissue infections. Neutrophils are having the following function in inflammation [28]

  - phagocytosis. Attachment of neutrophils to endothelium causing chemotaxis degranulation, killing foreign material.
  - Swallowing of antigen-antibody and nonmicrobial complexes.
  - Basement membrane destruction of the glomeruli[29] .

- EOSINPHILS

Eosinophls are fewer in number but it is bgger than neutrophils.Eosinophils constitutes of 1 -6% of white blood cells. Eosinophils are acid loving and distributes in medulla lower g.i.t ovary and lymph nodes. They mature in bone marrow and migrates to inflammatory sites of tissues. Eosinophils count is increased in Eosinophilia, Addisons disease Exfoliative skin diseases[30].

Eosinophils has the following functions

  - Eosinophils have a vital role in fighting against viral infections.
  - Eosinophils involve in the production of Elastase enzymes.
  - Production of cytokines
  - Production of Leukotriene and Eicosonoids mediators.

- BASOPHILS (MAST CELLS)

Basophils constitute about 0.01-0.3% of white blood cells. Basophil is seen in many inflammatory responses, mainly in allergic symptoms[31]. Heparin is present in basophils which is an anticoagulant. Basophils mature in bonemarrow .Histamine is released from basophils when it is activated.Basophils secrete LTD-4 and cytokines.

- LYMPHOCYTES

Lymphocytes is of three types Tcells ,B cells , Natural killer cells. Lymphocytes are mainly seen in lymph. They have large nucleus..Cell mediated immunity is mediated by T cells while humoral immunity is due to B cells. Natural killer cells has a vital role in protecting the host from tumours and virus. Release of cytotoxic granules from lymphocytes when they are activated. This helps in the destruction of altered cells. Lymphopoiesis is known as the formation of lymphocytes. Lymphocytes count is increased in viral infection, leukemias. Low T cell lymphocyte is seen in HIV[32].

- PLASMA CELLS

These cells are larger than lymphocytes with more abundant cytoplasm and an eccentric nucleus which has cart-wheel pattern of chromatin .Plasma cells are normally not seen in peripheral blood. They develop from B lymphocytes and are rich in RNA and α-globulin in their cytoplasm. There is an interrelationship between plasmocytosis and hyperglobulinaemia. These cells are most active in antibody synthesis.

Their number is increased in the following conditions:

Prolonged infection with immunological responses e.g. in syphilis, rheumatoid arthritis

- Tuberculosis
- Hypersensitivity states
- Multiple myeloma.

- **Mononuclear-Phagocyte System**[33]

It is located in Reticular connective tissue. Reticulo endothelial cell is mainly present in Spleen.This cell system includes cells derived from 2 sources with common morphology, function and origin These are as under:

Blood monocytes. These comprise 4-8% of circulating leucocytes.

Tissue macrophages.

These include the following cells indifferent tissues:

- Macrophages in inflammation.
- Histiocytes which are macrophages present in
- connective tissues.
- Kupffer cells are macrophages of liver cells.
- Alveolar macrophages (type II pneumocytes) in lungs.
- Macrophages/histiocytes of the bone marrow.
- Tingible body cells of germinal centres of lymph nodes.
- Littoral cells of splenic sinusoids.
- Osteoclasts in the bones.
- Microglial cells of the brain.
- Langerhans' cells/dendritic histiocytes of the skin.
- Hoffbauer cells of the placenta.
- Mesangial cells of glomerulus.

The mononuclear phagocytes are the scavenger cells of the body as well as participate in immune system of the body their functions in inflammation are as under:

Role of macrophages in inflammation. The functions of mononuclear-phagocyte cells are as under:

- Phagocytosis (cell eating) and pinocytosis (cell drinking).
- T lymphocytes release lymphokines by non immunologic which activates Macrophages a number of biologically active subastances .

- Proteases like collagenase and elastase which degrade collagen and elastic tissue.
- Plasminogen activator which activates the fibrinolytic system.
- Products of complement.
- Some coagulation factors (factor V and thromboplastin) which convert fibrinogen to fibrin.
- Chemotactic agents for other leucocytes.
- Metabolites of arachidonic acid.
- Growth promoting factors for fibroblasts, blood vessels and granulocytes.
- Cytokines like interleukin-1 and TNF-□.
- Oxygen-derived free radicals.

- GIANT CELLS

A few examples of multinucleate giant cells exist in normal tissues e.g. osteoclasts in the bones, trophoblasts in placenta, megakaryocytes in the bone marrow[34]. Macrophages fails to remove foreign bodies in chronic inflammation they fused to form a multi nucleated giant cells.multinucleated giant cells. Giant cells are of different types namely giant cell arteries, Langhans giant cells, Touton giant cells, Reed-Sternberg cell. It is seen in certain types of infection such as

H.I.V, Tuberculosis, Herpes

A. Giant cells in inflammation:

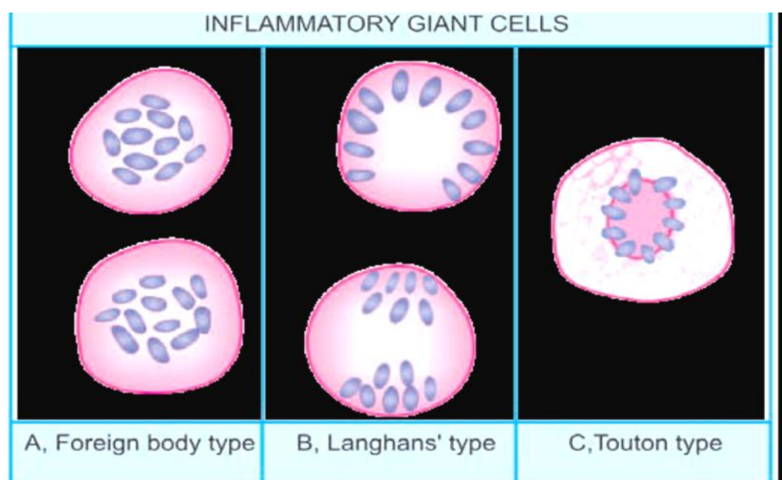
- Foreign body giant cells. It appears in response to a foreign body and is a collection of joined Giant cells and contains large number of nuclei of same size and are scattered in cytoplasam and are also seen in granulomatous infection likeleprosy and tuberculosis.

- Langhans' giant cells. It is seen in granulomatous conditions like tuberculosis and sarcoidosis[35]. These is a collection of epithelioid cells contains nuclei and the nuclei are arranged in the form of a ring .

- Touton giant cells. These multinucleated cells have vacuolated cytoplasm due to lipid content e.g. in xanthoma[36].It appears in high lipid content lesions such as Xanthoma, Dermatofibroma and Fat necrosis. It is multinucleated and identifies by the appearance of several nuclei and the nuclei are arranged like a ring surrounding homogenous cytoplasm. Cytokines involved in the formation of touton giant cells.

- Aschoff giant cells. These multinucleate giant cells are derived from cardiac histiocytes and are seen in rheumatic nodule[37]

Fig - 5 Inflammatory Giant Cells[2]

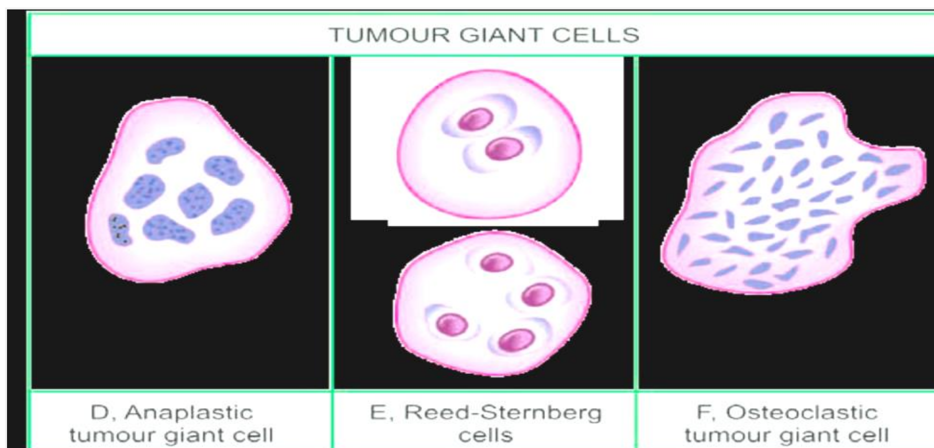


B. GIANT CELLS IN TUMOUR

Anaplastic cancer giant cells. These are larger, have numerous nuclei which are hyperchromatic and vary in size. Giant cells of various types.

- Foreign body giant cell with uniform nuclei dispersed throughout the cytoplasm.
- ‚Langhans' giant cells with uniform nuclei arranged peripherally or clustered at the two poles.
- Touton giant cell with circular pattern of nuclei and vacuolated cytoplasm.
- Anaplastic tumour giant cell with nuclei of variable size and shape.
- Osteoclastic tumour giant cell. and shape. These giant cells are not derived from macrophages but are formed from dividing nuclei of theneoplastic cells e.g. carcinoma of the liver, various soft tissue sarcomas etc.
- Reed-Sternberg cells. These are also malignant tumour giant cells which are generally binucleate and are seen in various histologic types of Hodgkin's lymphomas[38]. Giant cell tumour of bone. This tumour of the bones has uniform distribution of osteoclastic giant cells spread in the stroma.

Fig – 6 Tumour Giant Cells[2]



TREATMENT OF INFLAMMATION

The drugs used for treating inflammation and the associated pain is by NSAIDS, Opioid analgesic , Steroids, Antihistamines and combined use of analgesic and steroidal drugs. Salicylic acid was prepared by the hydrolysis of bitter glycoside obtained from willow bark. In 1875 sodium salicylate was used 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

Nonselective COX inhibitors

Salicylates	Aspirin
Propioniacid derivatives	Ibuprofen, Flurbiprofen, Naproxen
Anthranilic acid derivatives	Mephenamic acid
Aryl acetic acid derivatives	Diclofenac, Aceclfenac
Oxicam derivatives	Tenoxicam, Piroxicam, Meloxicam
Pyrollo pyrrole derivatives	Ketorolac
Indole derivatives	Indomethacin
Pyrazolone derivatives	Oxyphenbutazone, Phenylbutazone

PREFERENTIAL COX 2 INHIBITORS

Nimesulide, Nabumetone, Meloxicam

SELECTIVE COX 2 INHIBITORS

Celecoxib, Etoricoxib, Parecoxib

ANALGESIC ANTIPYRETICS WITH POOR ANTIINFLAMMATORY ACTION

Paracetamol, Metamizol, Nefopam

- OPIOID ANALGESICS

  NATURAL OPIOID ALKALOIDS

  Morphine, Codeine

  SEMISYNTHETIC OPIATES

  Pholcodeine,Diacetylmorphine

  SYNTHETIC OPIOIDS

  Tramadol, Methadone, Dextropropoxyphene, Fentanyl

- STEROIDAL ANTIINFLAMMATORY DRUGS

  NATURAL STEROIDS

  Cortisone

  Hydrocortisone

  SYNTHETIC STEROIDS

  Dexamethasone

  Betamethasone

  Triamcinolone

  Prednisolone

  Methylprednisolone
  Fludrocortisone

MECHANISM OF ACTION OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

The most important mechanism of action of anti-inflammatory action of NSAIDS is considered to be inhibition of prostaglandin synthesis at the site of injury. The anti-inflammatory action of different compounds is due to their ability to inhibit COX enzymes. Prostaglandins, Prostacyclin (PG I_2) and thromboxane A_2 (TXA_2) are produced from arachidoic acid by the enzyme cyclooxygenase namely COX1 and COX 2 respectively. Inflammation is the result of concerted participation of a large number of vasoactive chemotactive and proliferative factors. Activated endothelial cells express adhesion molecules on their surface and play a key role in directing circulating leucocytes to the site of inflammation. Some NSAIDS acts by additional mechanisms inhibition of activity of some of these molecules and generation of superoxide. Most NSAIDS inhibit COX1 and COX2 nonselectively but there are some selective COX 2 inhibitors

ADVERSE EFFECTS 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 transaminases, Hepatic failure (rare)

CNS

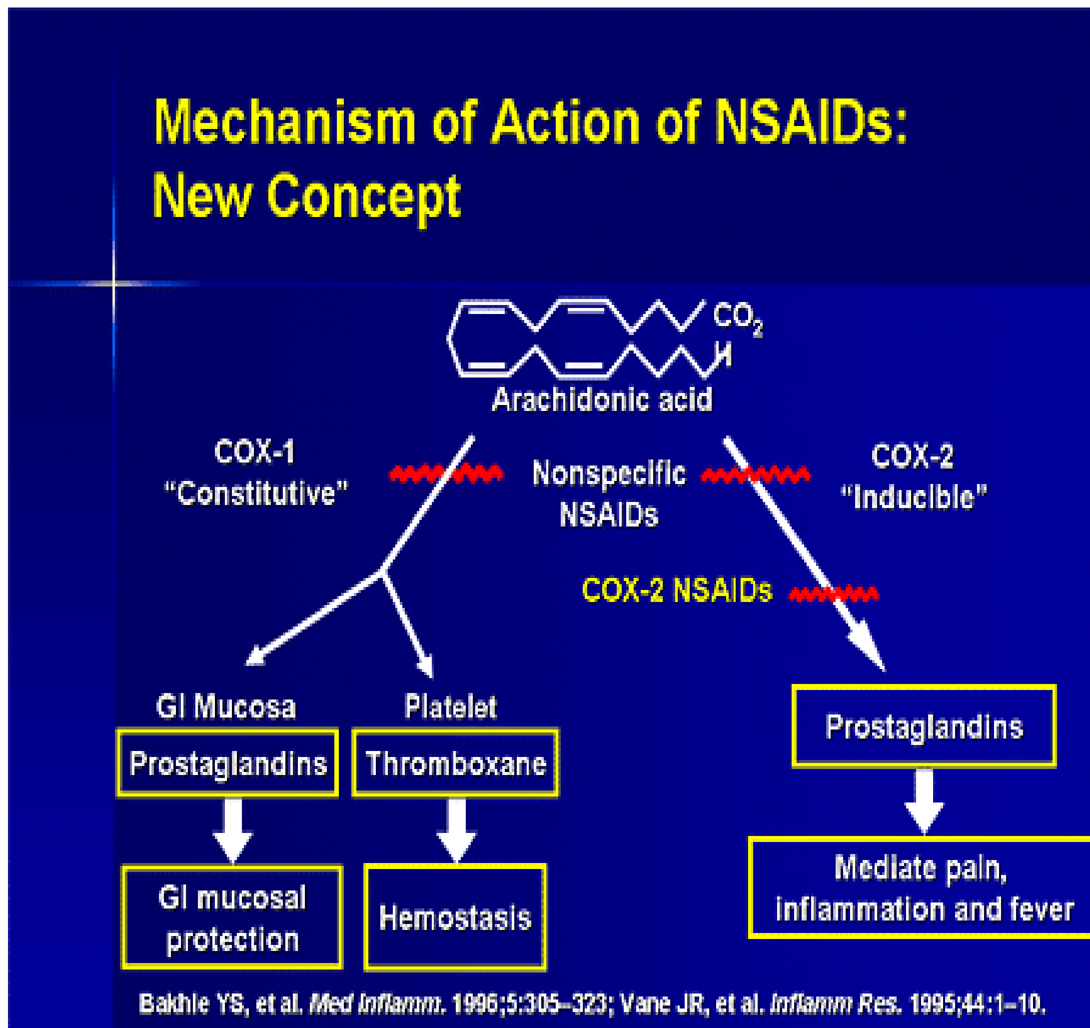
Headache, Mental confusion, Behavioural disturbaces, Seizure precipitation

HAEMATOLOGICAL

Bleeding, Thrombocytopenia, Haemolytic anaemia, Agranulocytosis

OTHERS :Asthma, Exacerbation, Nasal polyposis, Skin rashes, Pruritis, Angioedema

Fig-7 Mechanism Of Action Of NSAIDS[40]



MECHANISM OF ACTION OF OPIOIDS

Morphine and other opioids exert their actions by interacting with their specific receptors present on neurons in the CNS and in peripheral tissues. Opioid receptors are of three types mu , kappa and delta .Each has specific pharmacological profile and pattern of anatomical distribution in the brain spinalcord and peripheral tisssues. Opioid receptors are G protein coupled receptors located mostly on prejunctional 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 signale neurotransmitter release from 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.

Mu receptor

It is characterized by its high affinity for morphine .This is the main receptor mediating actions of morpine and its cogners. Endogenous ligands for mu receptor peptides are called Endomorphins 1 and 2 . High density of mu receptors has been detected in periaqueductal gray, thalamus, nucleus ambiguous.Two subtypes of mu receptors are also found; MU -1 and MU-2

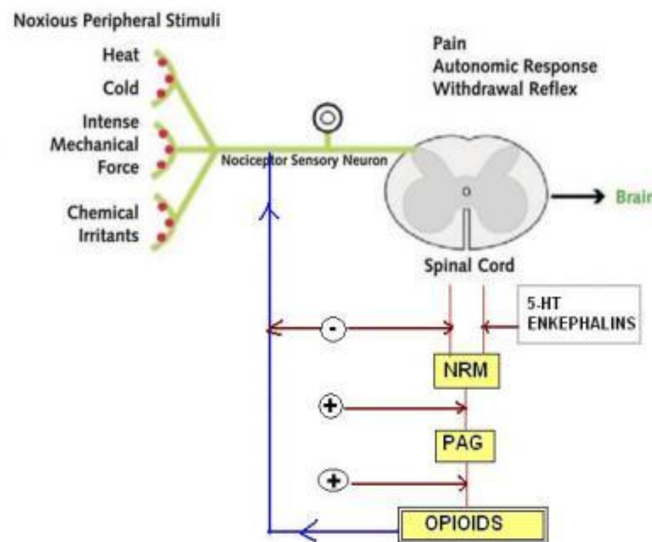
KAPPA receptor

It has high affinity for ketocyclazone and dynorphin. Two subtypes of kappa receptor are K_1 and K_3. Analgesia caused by kappa agonists is primarily spinal through K_1 receptor. K_3 receptors mediate lower ceiling supraspinal analgesia.

DELTA receptor

This receptor has high affinity for leu/met enkephalins. The delta mediated analgesia is mainly by spinal.

Fig 8 Mechanism Of Action Of Opioids[41]



ADVERSE EFFECTS 0F OPIOIDS

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

MECHANISM OF ACTION OF STEROIDS

The inflammatory response is suppressed by glucocorticoids.The action is non specific and covers all components and stages of inflammation reduction of increased capillary permeability local exudation cellular infiltration phagocytic activity capillary proliferation collagen deposition. The signs of inflammation such as redness swelling pain and heat is suppressed by steroids. Production of proinflammatory mediators like PG ,LT, PAF through inhibition of phospholipase A_2.. Decreased inducible PG ,IL production. Complement function is interfered. Prevention of tissue destruction.

ADVERSE EFFECT OF STEROIDS

Cataract formation

Osteoporosis

Hyperglycemia

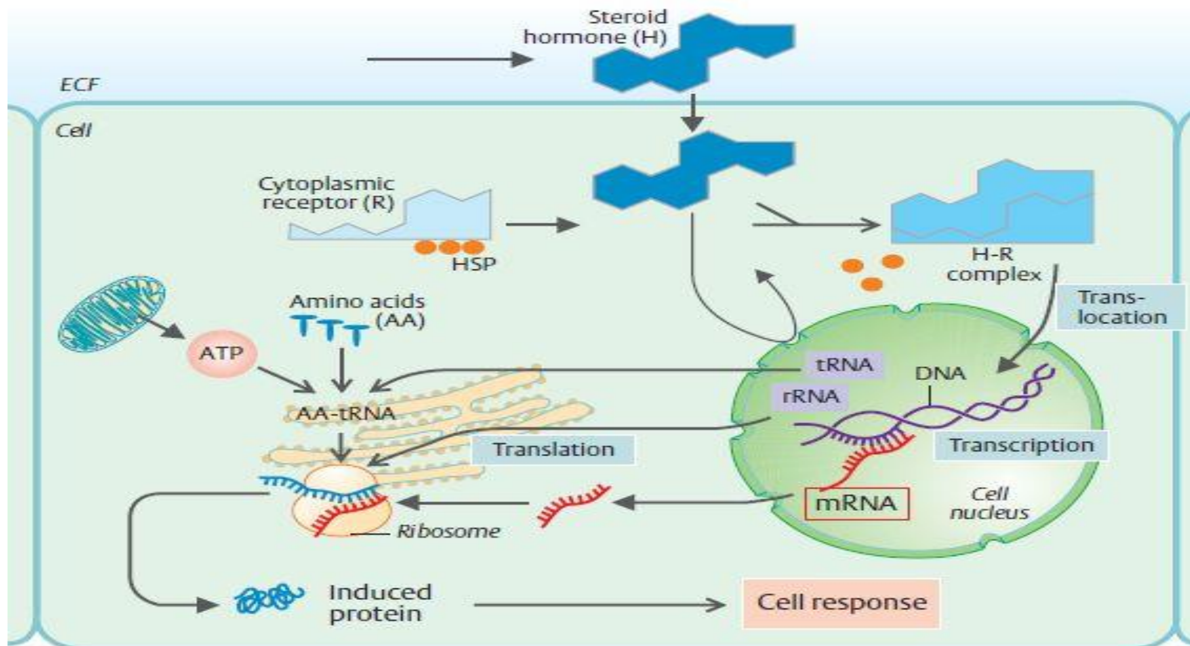
Muscle weakness

Delayed healing

Cushing syndrome

Peptic ulceration

Fig -9 Mechanism of Action of Steroids[42]



HERBAL MEDICINES HAVING ANTIINFLAMMATORY ACTION[43]

GINGER

Jamaican ginger used to treat dyspepsia, colic, rheumatoid arthritis,pain

TURMERIC

Research is ongoing for the effect of turmeric in disease like Alzheimers , Arthritis

DEVILS CLAW

It is used for its sedative , analgesic and diuretic properties

OTHER METHODS USED FOR TREATING INFLAMMATION

APPLYING ICE

For managing inflammation and pain this method is used by athelets

FISH OIL

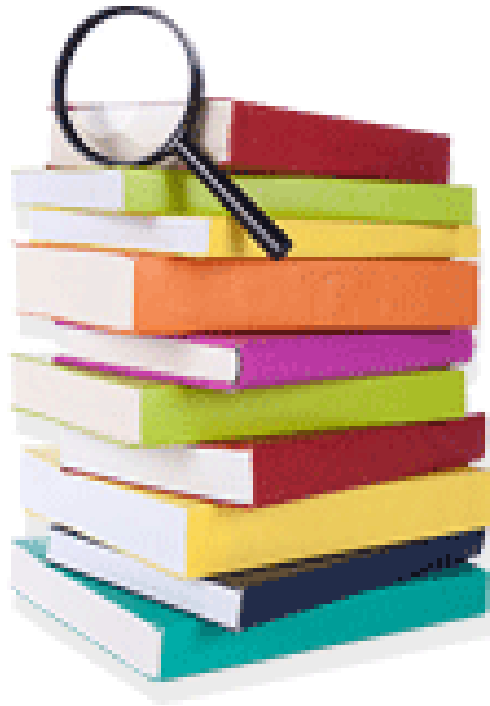
Reducing inflammation and anxiety as a result of daily consumption.

HERBAL MEDICATIONS AND THEIR USE

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. The utilization of natural substances has increased for various diseases amongst general public over the last few years not only because of their easy availability without prescription, cost and appointment to the health care professionals but also owing to the belief that natural substances has less adverse effects as compared to synthetic medicines.

According to World Health Organization the diabetic population is likely to increase up to 300 million or more by the year 2025.

Owing to the fact that there is a very significant community in the general public who always chose for alternative therapy than allopathic formulation, which makes it is clear that there is a global surge for a safe, efficacious, cost effective and easily available alternative drug for the long term management of many chronic diseases like diabetes, hyperlipidaemia etc. The current study is an attempt towards the search of such an alternative therapeutic agent



LITERATURE REVIEW

LITERTURE REVIEW[44-51]

Oludare et al., 2000. evaluated the anti-inflammatory effects methanolic extract of the Chromolaena odorata leaves in the carrageenan-induced rat paw edema model as well as for antipyretic activity in mice. The effects of the extract on intestinal transit of charcoal meal and castor oil induced diarrhoea were also investigated. The extract (50–200mg/kg) inhibited paw edema in rats and produced significant (p _0.05) reduction in rectal temperature of mice rendered hyperthermic by yeast suspension. Antimotility and antidiarrhoeal effects were produced by the extract in intact mice. This study reveals the anti-inflammatory, antipyretic and antispasmodic properties of C. odorata leaf.

Tran et al., 2011. identified the inhibitors of nitric oxide production and NF-αB activity from Chromolaena odorata (C. odorata). The compounds isolated from the aerial parts of C. odorata by bioassay-guided fractionation were investigated for their inhibitory effects on the NO production and NF-αB activity in LPS-stimulated RAW264.7 cells. Six fatty acids (S)-coriolic acid (1), (S)-coriolic acid methyl ester (2), (S)-15,16-didehydrocoriolic acid (3), (S)- 15,16-didehydrocoriolic acid methyl ester (4), linoleamide (5) and linolenamide (6) were isolated. All compounds inhibited the NO production at concentrations consistent with those required for NF-αB inhibition. Compound 2 was the most active with the IC50 values of 5.22 and 5.73 µM. The addition of a double bond in the fatty chain decreased the inhibitory effects while the methyl esterification increased the activities. Conclusions: The fatty acid components in C. odorata with NF-αB inhibitory activity could explain the anti-inflammation property of this plant in traditional medicine. This study could also contribute to the better use of C. odorata for human health care.

Elion et al., 2017. evaluated the anti-inflammatory and analgesic effects of the aqueous extract of leaves of *Chromolaena odorata* (Asteraceae) collected in Brazzaville-Congo. Acute inflammation was induced by using the carrageenan and formaldehyde models, and chronic inflammation by the cotton pellet induced granuloma model. Analgesic effect was evaluated by using the acetic acid-induced writhing, the pressure induced by the analgesymeter as well as the pain induced by formaldehyde. The results obtained show that aqueous extract (400 and 800 mg/kg) inhibits the edema induced by the carrageenan and formaldehyde. Moreover, this extract at the doses used (400 and 800 mg/kg) significantly inhibits granuloma fabric induced by cotton pellet. In addition, aqueous extract (400 and 800 mg/kg) inhibits significantly the pain induced

by the three methods used. In conclusion, aqueous extract of *C. odorata* has anti-inflammatory and analgesic effects. These observations justify the traditional use of this plant in the treatment of inflammatory pathologies and the pain.

Bamisaye et al., 2014. evaluated the ethano medical importance and phytochemical profile of Siam Weed (*Chromolaena odorata*) The aqueous leaves and roots extracts of the plant were studied to ascertain the nature of phytochemicals (secondary metabolites) present. This was with a view to provide the scientific basis for its use for the treatment of various ailments and diseases in these local government areas. The study combined the use questionnaire with laboratory study. Nine hundred and sixty five (965) respondents selected from these five local government areas participated in the study. Responses from the respondents revealed numerous ethno botanical uses of *Chromolaena odorata* in the management of many ailments and diseases. The phytochemical screening revealed the presence of flavonoids (0.067% leaves, 0.056% in roots), phenolics (0.076% in leaves, 0.091% in roots), saponins (0.016% in leave0.012% in roots), steroids (0.004% in leaves, 0.003% in roots) and tannins (0.054% in leaves a0.039% in roots). The screening did not reveal the presence of phlobatannins, cardiac glycosides, anthraquinones, cadenolides and dienolides and triterpenes in neither the leaves nor the roots of the plant. The presence of these secondary metabolites in *Chromolaena odorata* may likely form the basis of its effectiveness as ethno pharmacological plant among the Ijebus in Ogun State, Nigeria.

Fara et al., 2014. The study conducted investigates the allelopathic effects of *C. odorata* leaf extract on the germination and growth rate of *Vigna radiata* (mung bean). Methanolic extract at concentrations of 20, 40, 60 and 80% were used in the study in order to compare the effectiveness of allele chemicals level in inhibiting the growth of germinated mung bean. The methanolic extract from the leaves exhibited a potent growth inhibitory effect. Inhibition of roots and shoots elongation of mung bean showed a concentration and percentage methanolic extract dependent. Roots and shoots elongations were inhibited at all concentration of methanolic extract. Treatment with 80% methanolic extract showed a greater effect on germination rate of mung bean compared to other concentrations. Analysis was conducted on the 80% of methanolic extract to confirm the presence of alkaloids, tannins, flavonoids, steroids, terpenoids and carbohydrates compounds. The presence of tannins, flavonoids, alkaloids and terpenoids speed up the inhibition on mung bean growth as well as its germination.

Stanley et al., 2017. Evaluated the effect of ethanolic extract of *Chromolaena odorata* on the kidney and intestine of albino rats. Twenty growing albino rats with an average weight of 54 g were used in this study. They were grouped into four groups. First three known as the test groups, were given 50 mg/kg, 100 mg/kg, and 250 mg/kg ethanolic extract of *C. odorata*, respectively, while the control group (4) was given distilled water orally. The experiment was performed for 6 weeks. The animals were killed using chloroform suffocation. The kidneys and the intestine were harvested and fixed in 10% neutral buffered formalin for histological analysis. Blood samples were collected from the animals by heart puncture for estimation of creatinine and urea levels. The creatinine, urea, aspartate aminotransferase, alanine transaminase, and alkaline phosphatase levels of blood sample from the test group were significantly different when compared with the control ($p < 0.05$). The histological sections of the kidneys in this study showed no signs of degeneration. Infiltration of inflammatory cells and epithelial erosion were observed in the histology sections of the intestine of all the test groups. The results revealed that uncontrolled use of this plant extract has an adverse effect on the kidney function and on the histology of the intestine of the rats used in this study.

Hataichanok et al., 2013. Chromolaena odorata (L.) examined the molecular mechanisms by which Siam weed extract (SWE) affected hemostatic and wound healing activities. SWE promoted Balb/c 3T3 fibroblast cell migration and proliferation. Subsequently, we found that heme oxygenase-1 (HO-1), the accelerating wound healing enzyme, was increased at the transcriptional and translational levels by SWE treatments. The HO-1 promoter analyzed with luciferase assay was also increased by treatment of SWE in a dose-dependent manner. This induction may be mediated by several kinase pathways including MEK, p38MAPK, AKT, and JNK. Quantitative real-time PCR using undifferentiated promonocytic cell lines revealed that thromboxane synthase (TXS), a potent vasoconstrictor and platelet aggregator, was increased and MMP-9, an anti-platelet aggregator, was decreased in the presence of SWE. The study presented that, SWE accelerated hemostatic and wound healing activities by altering the expression of genes, including HO-1, TXS, and MMP-9.

Okpashi et al., 2014. Investigated the possibility of using Chromolaena odorata to inhibit bacterial and fungal growths. The antimicrobial effects of independence leaves (Chromolaena Odorata) siam weed obtained from Bumaji in Boki local government area of crosss river state were studied. An ethanolic extract of the dried and coarse leaves was prepared, serial dilutions of the extracts 100%, 50%, 25%, 12.5%, 6.2%, 3.13% and 1.5% respectively were carried out in water for all the drugs and extracts. They were tested for sensitivity and resistivity on bacterial such as Escherichia coli, Saimonella, Staphylococcus aureus and Bacilus anthracilis, using penicillin, zinacef, ciprofloxacin, ampicillin and ceftriaxone as standard controls. The serial dilutions were also tested on fungi such as candida albicans and tricophyton tonsurans, while clotrimazole and nystatin were used as standard control for antifungals. Chromolaena odorata was observed to exhibit concentration- dependent antibacterial effects similar to control bactericidal agents and antifungal effects similar in pattern to the control drugs used in this research. C. odorata extracts could thus possess antibacterial and antifungal ingredients.

PLANT PROFILE[52-70]

CHROMOLAENA ODORATA PLANT

FLOWERS

LEAVES

ROOTS

CHROMOLAENA ODORATA

Common Names

Armstrong's weed, baby tea, bitter bush, butterfly weed, Christmas bush, chromolaena, devil weed, eupatorium, Jack in the bush, Jack-in-the-bush, kingweed, paraffinbush, paraffinweed, Siam weed, turpentine weed, triffid weed

Taxonomical Classification

Kingdom	*Plantae*
Subkingdom	*Viridiplantae*
Division	*Tracheophyta*
Subdivision	*Spermatophytina*
Class	*Magnoliopsida*
Order	*Asterales*
Family	*Asteraceae*
Genus	*Chromolaena*
Species	*C. odorata.*

Plant Description

Chromolaena Odorata has a minimum 10-year life span. *It* is a scrambling perennial shrub which grows 2–3 m in height with straight, pithy, brittle stems that branch readily. The plant genus *Chromolaena* is a genus of the family *Asteraceae* which comprises over 165 species that are distributed across tropical and subtropical regions. The name is derived from the Greek word meaning "color." Due to its species name "*odorata*,"

Stems and Leaves: The slender stems are generally yellowish-green and somewhat hairy (i.e. pubescent), but become woody towards the base of the plant. These stems grow up to 7 m or more in length and several are usually produced from the plants long-lived root-stock (i.e. crown). They are much-branched, with the side (i.e. lateral) branches usually being produced in pairs in the leaf forks (i.e. axils).

The oppositely arranged leaves (5-12 cm long and 3-7 cm wide) are triangular or egg-shaped in outline (i.e. ovate) and have a pointed tip (i.e. acute apex). They are hairy (i.e. pubescent) on both surfaces and have coarsely toothed (i.e. serrated) margins. These leaves are borne on stalks (i.e. petioles) up to 6 cm long (usually 10-15 mm), and give off a strong odour when crushed.

Flowers and Fruit: The small flower-heads (i.e. capitula) do not have any 'petals' (i.e. ray florets) and are borne in dense clusters at the ends of the branches (i.e. in terminal panicles). These flower-heads (about 10 mm long and 3 mm wide) are pale pink or pale mauve in colour (sometimes appearing whitish when older) and consist of numerous (15-30) tiny flowers (i.e. tubular florets). These tiny flowers (10-12 mm long) are surrounded by several layers of overlapping slender bracts (i.e. an involucre) 8-9 mm long. Each flower-head (i.e. capitulum) is borne on a stalk (i.e. peduncle) 10-30 mm long. Flowering occurs from late summer through to early spring, but is most abundant during winter.

The black or dark brown 'seeds' (i.e. achenes) are 4-5 mm long and topped with a ring (i.e. pappus) of white to brownish coloured hairs (5-6 mm long).

Roots: The roots are narrow and fibrous and generally reach 0.3 km in depth. *C. odorata* shows morphological in terms of flower color, leaf shape, odor of the crushed leaves, and plant architecture variable in its native environment.

Distribution

C. odorata is often noted as a native of tropical Central and South America, from Mexico and the Caribbean to Brazil; however, the exact northern and southern limits of its native range remain uncertain and are likely to also include some regions outside of the tropics. USDA-ARS (2007) include as part of the native range Texas and Florida (USA), and all South American countries except Chile and Uruguay and records from Córdoba in Argentina suggest latitudinal limits of approximately 30° North and South. Gautier (1992b) included Uruguay as part of the native range and USA populations as introduced, neither being confirmed in later studies.

Phytochemical Substances

The dried leaf of *C. odorata* contained ash (11%), crude fat (11%), fiber (15%), moisture (15%), crude protein (18%), and carbohydrate (31%).Its active phytochemical substances are as follows: flavonoid aglycones (flavanones, flavonols, flavones) including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin; terpenes and terpenoids; essential oils; alkaloids including pyrrolizidine, saponins and tannins; phenolic acids (including ferulic acid, protocatechuic acid); phytoprostane compound including chromomoric acid.

USES

Literatures suggest that *C. odorata* possess anti-bacterial, anticancer, anticonvulsant, antidiabetic, anti-diarrheal, anti-fungal, anti-inflammatory, antioxidant, antiparasitic, hemostatic, wound healing, and hepatoprotective activities.

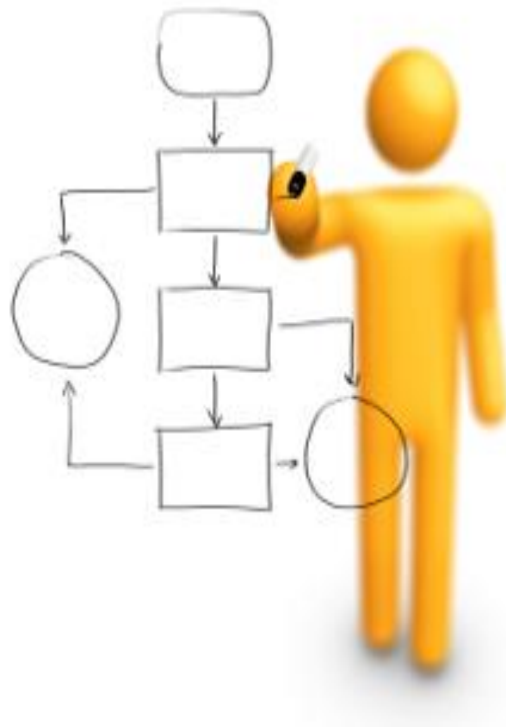
AIM & OBJECTIVES

AIM:

To evaluate the toxicity profile, analgesic and anti-inflammatory activity of ethanolic extracts of *chromolaena odorata* root on rat models.

OBJECTIVE OF THE STUDY:

1. To successfully extract the root of *chromolaena odorata*.
2. To fix the effective and lethal dose of the extract using acute and sub-accute toxicity studies.
3. To evaluate the analgesic and anti-inflammatory activity of the *chromolaena odorata* root extract using rat models.
4. To successfully document the results and comparing using specific statistical tools.



MATERIALS & METHODS

5. MATERIALS AND METHODS

Plant material

The root samples of *chromolaena odorata* were collected from the herbal store and botanical garden of The Coimbatore Ayurveda college, Coimbatore. The plant was identified and authenticated by comparison with herbarium specimens.

The dried weighed coarse powder was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents.

Animals

Wistar rats (150 – 250 g) used for the study were obtained from the animal house of the Department of Pharmacology, RVS College Of Pharmacy, Coimbatore. The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. The animals were housed three per cage in a polypropylene cage and maintained in standard laboratory conditions with free access to food and water *ad libitum*[71]. All animal experiments were conducted in compliance with (Organization for Economic Cooperation and Development) OECD Guideline and approved by the Institutional Animal Ethics Committee, RVS College of Pharmacy.

Chemicals, Drugs and Instruments

Indomethacine, carrageenan and anesthetic ether were procured from a chemical store at Coimbatore (Ponmani and Co). Other important chemical used in phytochemical analysis like alcohol, hydrochloric acid, ∞- napthol, Sulphuric acid, Fehling A&B, Benedict reagent, sodium hydroxide, nitric acid, ammonia, lead acetate, ninhydrin, sudan red III reagent, glycerin, picric acid, chloroform, acetic anhydride, ferric chloride, zinc, dragendroff's reagent, Wagner's reagent, Mayer's reagent, sodium chloride and bromin water were collected from the store of RVS College of Pharmacy. All the chemicals used in the study are of analytical grade.

II EXTRACTION[72]

The roots of *chromolaena odorata*, was dried under shade and are carefully grinded using a blender. The coarse power so obtained was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents. The assembly of Soxhlet apparatus is as shown in the figure**.**

Petroleum ether extract

About 500gm of coarse powder was extracted with 2.5litre of petroleum ether (60-80°C) by continuous hot percolation using Soxhlet apparatus. The extraction was continued up to 24hours.After completion, the petroleum ether was extract was filtered and solvent removed by distillation under reduced pressure. The obtained residue was stored in a dessicator.

Chloroform extract

Marc obtained from the above extract was dried, extracted with 2.5 of chloroform (79-81°) by using Soxhlet apparatus. The extraction was continued up to 24 hours. After completion of extraction, the chloroform was filtered and solvent removed by distillation under reduced pressure. Then it was stored in dessicator.

Acetone extract

Marc obtained from the extract was dried, extracted with 2.5litre of acetone by Soxhlet apparatus. The extraction was continued up to 24 hours. After completion of extraction, the acetone extract was filtered and solvent removed by distillation under reduced pressure. Then the extract was stored in a dessicator.

Ethanol extract

Marc obtained from the above extract was dried and extracted with 2.5litre of Ethanol. Then it was filtered and stored in dessicator.

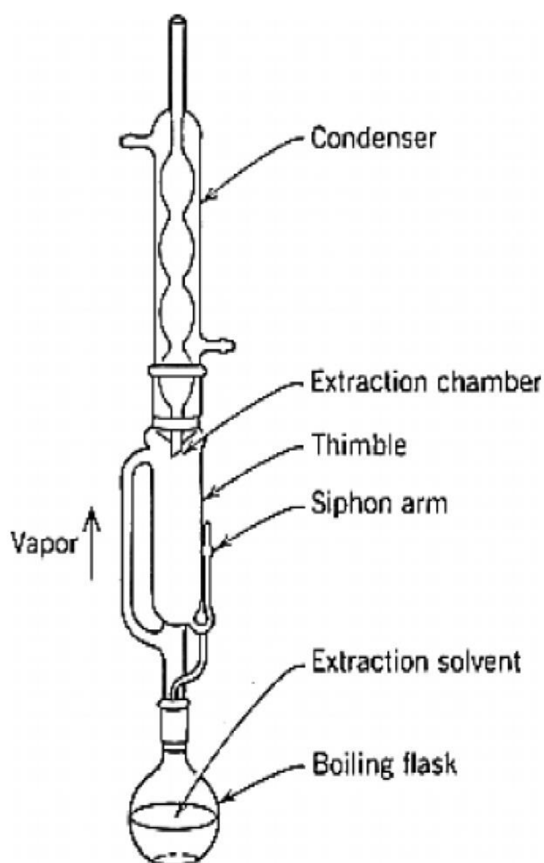
Aqueous extract - Cold maceration

Marc left behind after alcoholic extraction was taken and finally the marc was macerated with 3 liters of chloroform water (0.25%) in a narrow mouthed bottle for 3 days. After the completion

of extraction it was filtered. The solvent was then removed by distillation under reduced pressure. The extract was then stored in dessicator.

From the weight of each extractive residue the values of extractive residue is calculated in percentage. All the above extracts were used for identification of constituents by preliminary phyto chemical tests and pharmacological screening.

Image 01 – Extraction using soxhlet apparatus assembly



II- PHYTO CHEMICAL SCREENING[73-74]

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phyto constituents are the contributors of pharmacological activities of a plant.

TEST FOR CARBOHYDRATES

Molish test

The sample powdered was added with 1 ml of alpha napthol solution along with conc Sulphuric acid solution in the test tube reddish colour was produced at the junction between 2 liquid this is shows the presence of carbohydrate.

Fehling test.

To the sample powder was added with both Fehling A and Fehling B solution and placed in the water bath for a sufficient time. This shows the brick red colour. It shows the presence of carbohydrate.

Benedicts test.

To the sample powder add 8 drops of benedict's reagents and Boil the sample vigorously for 5 min it shows the red ppt. this shows the presents of carbohydrate.

TEST FOR ALKALOIDS

To the small portion of stored powder (sample) was taken and add few drops of hydrochloric acid and filtered. The filtered was tested with various alkaloid agents.

Mayer's reagents:

To a small portion of the above filter add small quantity of Mayer's reagent to form cream precipitate. This shows the presence of alkaloids.

Dragendorffs reagents

From the above filter add small amount of Dragendorffs reagents it forms a orange brown precipitate. This shows the presents of alkaloids.

TEST FOR FLAVONOIDS

To the filter of the plant extract add 5 ml of dilute ammonia solution and followed by the addition of concentrated sulphuric acid. It forms a yellow colour. It shows extract indicated the presence of flavonoids.

TEST FOR STEROIDS.

Salkowaski test

Few amount of plant extract was mixed with chloroform and the same volume of sulphuric acid is added on it. Cherry red colour was obtained in the chloroform layer. This shows the sample contain steroids.

Libbbermann burchatd test:

The extract is dissolved in 2 ml of chloroform 10 drops of acetic acid and conc. Sulphuric acid were added. Now the solution becomes reddish colour then it turns to bluish green colour. This shows the plant extraction indicates the presents of steroids.

TEST FOR TANNINS.

From few amount of plant extract is treated with vanillin hydrochloric acid reagent. It forms, pink or red colour due to the formation of phloroglucinol, it indicate the presence of tannins.

TEST FOR PROTEIN.

Mellon’s reagents.

Mellon’s reagents (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

Ninhydrin Test.

To the sample solution, 2 drops of freshly prepared 0.2% ninhydrine reagent was added and heated. Development of blue colour may indicate the presence of peptide, amino acid (PROTEIN).

TEST FOR GLYCOSIDES:

Keller- killani test.

From the small quantity of small powder acetic acid was dissolved and adds few drops of ferric chloride and transferred to the surface of conc Sulphuric acid. At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presents of cardiac glycosides.

TEST FOR SAPONINS.

Foam test:

1 ml of extract solution is diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of Saponins.

IV TOXICOLOGICAL STUDIES[760]

COLLECTION AND AUTHENDICATION:

Chromolaena odorata root(Asteraceae) were collected form the local source ,comibatore,Tamilnadu the month of February.The plant material was identified and authenticated by prof.P.Jayaraman Ph.D, Director.

Animal Ethical Committee approval

Animal experiment was performed asper the protocol by the institutional animal ethicals committee RVS COPS/IAEC/2019/007.

ACUTE TOXICITY STUDY

Experimental Protocol:

Guideline	:	OECD-423[75]
CPCSEA Ref. No	:	IAE1012/c/17/CPCSEA
Test	:	Limit test
Species	:	*Rattus norvegicus*
Strain	:	Albino Wistar rats
Number of animals	:	24 animals (6 for each group)
Sex	:	Female
Initial dose	:	5mg/kg
Route of administration	:	Oral
Duration	:	3hr close observation, followed by 14 days observation
Others	:	Body weight, mortality status
Parameters	:	CNS, ANS and behavioural changes
Blood collection	:	Not needed
Sacrifice	:	Not needed

Selection of Test animals.

Female adult Wistar rats of 8-12 weeks are selected. Nulliparous and non-pregnant animals were obtained from the centralized animal house of RVS College of Pharmaceutical sciences, Coimbatore and they are acclimatized for holding 1 week prior to dosing.

Housing and feeding conditions for Experimental Animals.

Temperature - As per OECD guideline-420 the temperature of animal house were maintained at $22^0C \pm 3^0C$.

Humidity - The relative humidity of animal room maintained at 50-60% preferably not exceeds 70% (OECD guidelines-420, 2001). Otherwise there may be chances of developing lesions such as ring tail and food consumption may be increased.

Light – The sequence of light used was 12 hrs light and 12 hrs dark.

Caging – Polypropylene cages with solid bottom and walls. The lids are made up of stainless steel grill which is capable to hold both feed and water.

Feeding condition and feed – Sterile laboratory feed (*ad libitum*) and water daily. The feed used were brown coloured chow diet.

Drug administration

Animals are fasted prior to dosing (food but not water should be withheld for overnight).After that animals are weighed and the test substance was administered. The healthy rats has been taken and divided into 4 different groups. The test substance was administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle.

Experimental Design

In this study, 4 groups of 6 rats each were given 5, 50 and 300 and 2000 mg/kg of the extract (p.o.). After drug administration the food is withheld for 3 hours. The animals are observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days, post treatment to observe for any symptoms of toxicity and mortality. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), autonomic effects (salivation,

lacrimation, gauntness and piloerection) and central nervous system (gait, tremors and convulsion) were carried out and changes were noted (OECD, 2001).

Table: 1 Experimental Designs.

Group	Dose(mg/kg)
Group I	5
Group II	50
Group III	300
Group IV	2000

Clinical observation

All animals were monitored continuously with special attention for 4 hrs after dosing for signs of toxicity. Additional observations are also done for the next 14 days for any other behavioural or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed. LD_{50} values are established using the formula.

Dose Calculation Equation[76]

$$\mathbf{LD_{50} = higher\ dose - \Sigma\ (a \times b)/n}$$

Where,

a = dose difference

b = animal died

n = No. of animals in each group

$$\mathbf{ED_{50} = \frac{LD_{50}}{10}}$$

SUB-ACUTE TOXICITY STUDY

Experimental Protocol:

Guideline	:	OECD-407 [77]
CPCSEA Ref. No	:	IAE1012/c/17/CPCSEA
Species	:	*Rattus norvegicus*
Strain	:	Albino Wistar rats
Number of animals	:	6 for each dose
Sex	:	Male/Female
Route of administration	:	Oral
Duration	:	28 days
No. of blood collection	:	2
Duration of blood collection	:	0th day, 28th day
Blood collection route	:	Retro orbital
Sacrifice	:	After 28 days of oral administration
Body weight recording	:	weekly intervals

Selection of Animals

Male and Female rats were selected and are acclimatized for 5 days prior to the start of study. The females rats should be nulliparous and non-pregnant. At the commencement of study the weight variation of animals used minimal and not exceed ± 20 % of the mean weight of each sex. Repeated dose oral study was conducted as a preliminary to a long term study preferably animals from the same strain and source were used in both studies.

Housing and feeding conditions

The temperature in the experimental animal room was maintained at 22^{0}C (±3^{0}C). The relative humidity was 50-60% (not exceed 70%) and the lighting sequence was 12 hrs light and 12 hrs dark. For feeding, conventional laboratory diet was used with an unlimited supply of drinking water. Animals were housed in small groups of same sex (NMT 5 animals in each cage)

Preparation of animals

Healthy young adult animals were randomly assigned to control and treatment groups. Cages were arranged in such a way that possible effects due to cage placement were minimized. The animals were identified uniquely and kept in their cages for five days prior to the start of the study to allow for acclimatization to the laboratory conditions.

Experimental Design

Twenty four wistar rats were divided into four groups of six (3 male and 3 female) rats each. Three different doses viz, 0, 200 mg/kg, 500 mg/kg and 1000 mg/kg were selected for the study. The doses are selected according to the OECD Guideline 407. Group 1 served as the control and received 4 ml/kg of distilled water while groups 2-4 received 200 mg/kg, 500 mg/kg and 1000 mg/kg of the extract for 28 days. All the rats were observed for any physiological and behavioral changes and mortality.

Table 02 - Experimental Design of Sub-Acute Toxicity Study

GROUP	Number of Animals	DOSE (mg/kg)
Group 1 (Control)	6 (3+3)	0
Group 2	6 (3+3)	200
Group 3	6 (3+3)	500
Group 4	6 (3+3)	1000

Statistical Analysis.

Statistical comparison was performed using one way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. All statistical analysis was performed using SPSS statistical version 17.0 software package (SPSS Inc., USA).

ANALGESIC ACTIVITY [78-87]

Analgesic Activity of Ethanol Extract of *Chromolaena Odorata* Using Eddy's Hot Plate Method in Mice

Swiss albino mice of either sex (20 to 25 gm) were obtained orginally from the centralized animal house of RVS College of Pharmaceutical sciences, Sulur. Animals had free access to standard diet and maintained under environmental conditions. They were housed in animal cages with 10/14 h light/dark cycle in an air conditioned area at 25 ± 2∘ C. Depending on pilot experiments using six animals per each group the optimum conditions for experiments were decided. The standard control used was Indomethacin.

Image 2: Eddy's Hot Plate

Procedure

In this experiment, four groups (n=6) of Swiss albino mice (20–25 g) were placed on a hot plate maintained at 55±1°. . The reaction of animals such as paw licking or jump response is taken as as the endpoint. A cut-off time 15 seconds is followed to avoid any thermal injury to the paws. Substance which is having analgesic effect increase the reaction time. Food was withdrawn on the preceding night of the experiment day. Group-1 normal control (Normal saline 10ml/kg/ p.o.),group-11 Standard Group -Indomethacin(5mg/kg/po.), whereas groups-3 and 4 animals received ethanolic extracts of ***Chromolaena Odorata*** (200 and 400 mg/kg, p.O) respectively.

Each animal was then individually placed gently on Eddy's hot plate at 55°C. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate, were determined 15, 30, 45, 60, and 90 min after administration of the test drug or vehicle (Jeane Silva et al., 2003).

Table 03 - Experimental Design of Eddys Hot Plate Experiment.

GROUP	Number of Animals	DOSE
Group 1 (Normal Saline)	6 (3+3)	10 ml/kg
Group 2 (indomethacin)	6 (3+3)	5 mg/kg
Group 3 (extract)	6 (3+3)	200 mg/kg
Group 4 (extract)	6 (3+3)	400 mg/kg

Analgesic Activity of Ethanol Extract of *Chromolaena Odorata* Using Tail Flick Method[68].

Wister albino rats of either sex (150 to 200 gm) were obtained originally from centralized animal house of RVS College of Pharmaceutical sciences, Sulur. Animals have free access to standard diet and maintained under environmental conditions. They were housed in animal cages with 10/14 h light/dark cycle in an air conditioned area at 25 ± 2∘ C. Depending on pilot experiments using six animals per group the optimum conditions for experiments were decided. The standard control used was Indomethacin.

Procedure

Tail flick test was assessed by using an analgesiometer. Rats were divided into 4 groups each of having six animals. Control (group 1) receiving Normal Saline 10 ml/kg, Standard reference group (group 2) receiving Indomethacin (5 mg/kg) while group 3 and group 4 are receiving ethanol extracts of ***Chromolaena Odorata*** root (200 and 400 mg/kg) orally. The tip of the rat was placed over the aperture. The animal responded to the focused heat stimulus by flicking its inflicted tail. The reaction time was recorded at 30, 60, 120, 180 minute respectively after the administration of the test standard and control. The test was terminated at 10 seconds to prevent tissue damage. The time taken by the rats to withdraw the tail was taken as the reaction time

Image 3: Analgesiomete used in the Tail Flick Experiment.

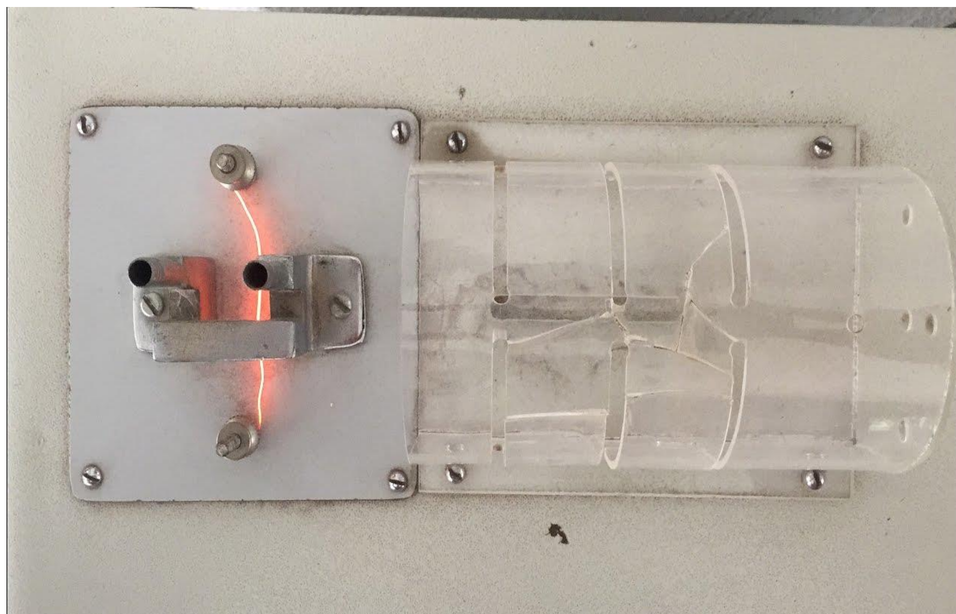


Table 04 - Experimental Design of Tail Flick Experiment.

GROUP	Number of Animals	DOSE
Group 1 (Normal Saline)	6 (3+3)	10 ml/kg
Group 2 (indomethacin)	6 (3+3)	5 mg/kg
Group 3 (extract)	6 (3+3)	200 mg/kg
Group 4 (extract)	6 (3+3)	400 mg/kg

ANTI INFLAMMATORY ACTIVITY.[88-90]

Anti-Inflammatory Activity of Ethanol Extract of *Chromolaena Odorata* Using Carrageenan Induced Paw Edema in Rats.

Wister albino rats of either sex (150 to 200 gm) were obtained originally from centralized animal house of RVS College of Pharmaceutical sciences, Sulur. Animals have free access to standard diet and maintained under environmental conditions. They were housed in animal cages with

10/14 h light/dark cycle in an air conditioned area at 25 ± 2∘ C. Depending on pilot experiments using six animals per each group the optimum conditions for experiments were decided. The standard control used was Indomethacin.

Procedure

Rats were divided into 4 groups each of having six animals. Control group (group 1)receiving Normal Saline 10 ml/kg, Standard reference group (group 2) receiving Indomethacin (5 mg/kg) while group 3 and group 4 are receiving ethanol extracts of ***Chromolaena Odorata*** roots (200 and 400 mg/kg) administered orally 30 minutes prior to carrageenan injection. Carrageenan was injected in the subplantar region of the right paw. Acute inflammation was experimentally induced half an hour after subplanter injection of carrageenan (0.1 mL freshly prepared 1% suspension) in right hind paw of rats . By using plethysmographic method paw volume was measured initially and at 1, 2, 3, and 4 h after carrageenan treatment.

Image 4: Plethysmograph and carrageenan administration

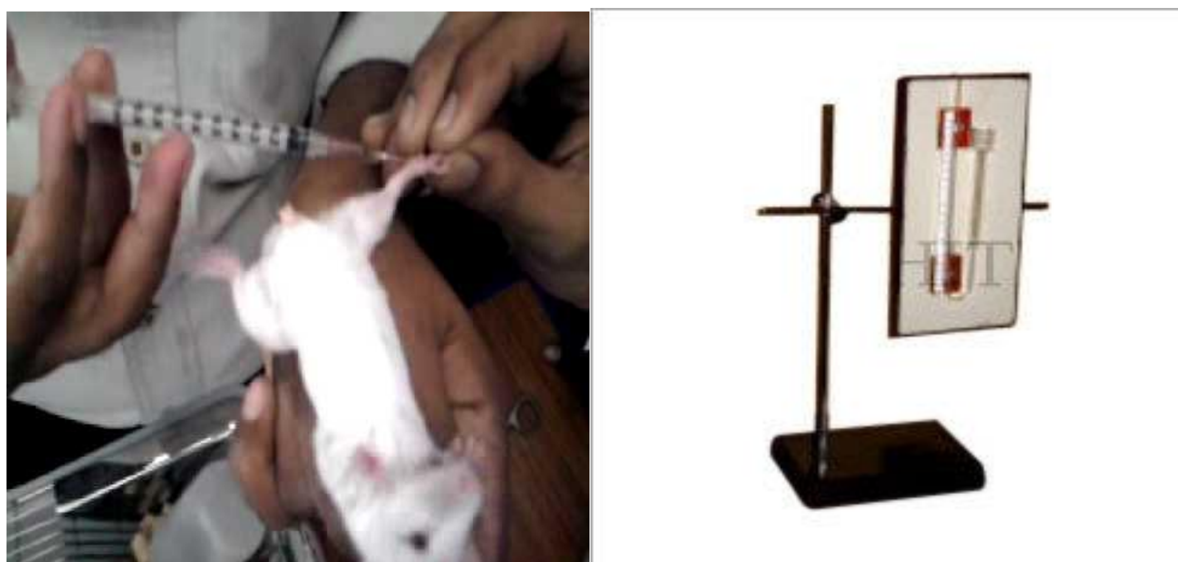


Table 05 - Experimental Design of Carrageenan Induced Paw Edema Experiment.

GROUP	Number of Animals	DOSE
Group 1 (0.1ml Carrageenan + Normal Saline)	6 (3+3)	10 ml/kg
Group 2 (0.1ml Carrageenan +	6 (3+3)	5 mg/kg

indomethacin)		
Group 3 (0.1ml Carrageenan + extract)	6 (3+3)	200 mg/kg
Group 4 (0.1ml Carrageenan + extract)	6 (3+3)	400 mg/kg

Anti-Inflammatory Activity of *Chromolaena Odorata* ethanol Extract Using Cotton Pellet induced granuloma in Rats.

Male Wister rats of either sex (150 to 200 gm) were obtained originally from centralized animal house of RVS College of Pharmaceutical sciences, Sulur. Animals have free access to standard diet and maintained under environmental conditions. They were housed in animal cages with 10/14 h light/dark cycle in an air conditioned area at 25 ± 2∘ C. Depending on pilot experiments using six animals per each group the optimum conditions for experiments were decided. The standard control used was Indomethacin.

Procedure

Rats were divided into 4 groups each of having six animals. Animals are anaesthetized with ether. Cotton pellets of weight (5mg) each were sterilized or either standardized for use in dentistry are used. Under ether anaesthesia the cotton pellets were introduced subcutaneously through a skin incision on the back of the animal. 30 minutes after the implantation of cotton pellets, Control group (group1)Normal Saline 10 ml/kg, Standard reference group (group2) Indomethacin (5 mg/kg) while group 3 and group 4 have ethanol extracts of ***Chromolaena Odorata*** roots (200 and 400 mg/kg) administered orally for 7 days. On the 7th day, the animals were sacrificed. The granulomatous tissue with cotton pellet was removed and dried at 60°C to constant weight.The dry weight of the cotton pellet was taken. Weight of the cotton pellet before implantation was subtracted from the weight of the dried cotton pellets. Dried weight of the

granulomaformed was used for the statistical analysis. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

Stastical Methods

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS

RESULTS

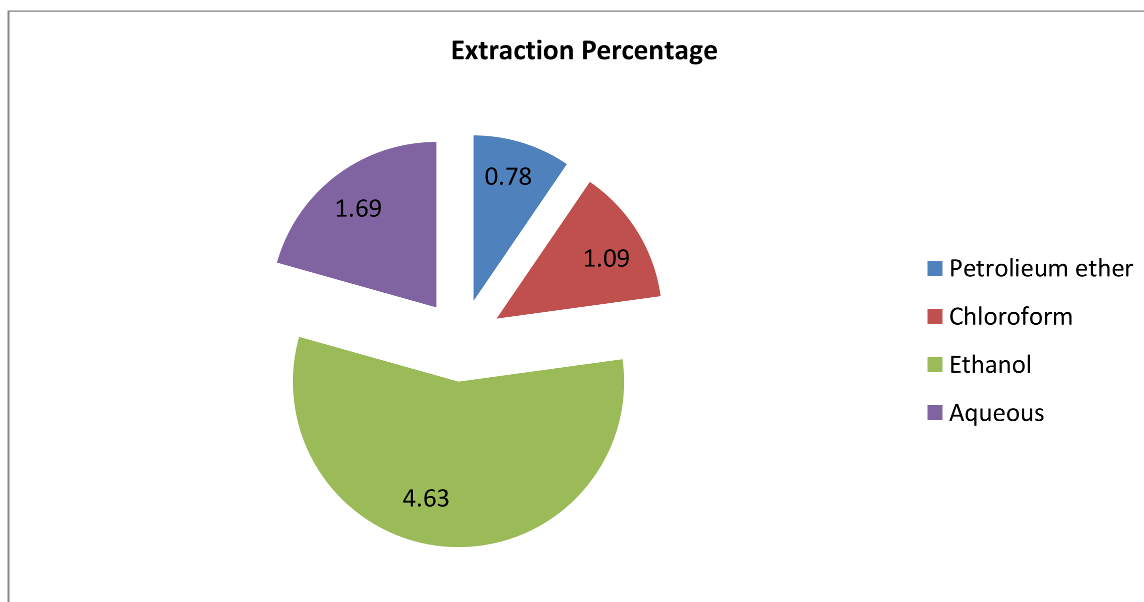
SOXHLET EXTRACTION OF *CHROMOLAENA ODORATA*

The percentage yield of various extracts viz. petroleum ether (60-80°C), chloroform and ethanol and water was 0.78%, 1.09%,4.63%, 1.69% w/w respectively.

Table 6: Soxhlet Extraction of *Chromolaena Odorata*

Plant	Part used	Method of Extraction	Solvents	Percentage Yield (%W/V)
Chromolaena Odorata	Dried Root	Continuous Hot percolation by Soxhlet apparatus	Petroleum ether(60-80°C)	0.78
			Chloroform	1.09
		and aqueous extraction by Cold maceration	Ethanol	4.63
			Aqueous	1.69

FIG: 6- Composition of Avarage Value of Extractives



PRELIMINARY PHYTO CHEMICAL SCREENING.

Chromolaena Odorata extracts was subjected to various chemical tests as per the standard methods for the identification of the various constituents. The result of this phyto chemical analysis is listed below.

Table 7 - Qualitative Phyto-Chemical Screening of ***Chromolaena Odorata Extracts***

PLANT CONSTITUENT	INFERENCE			
	Pet ether extract	**Chloroform extract**	**Ethaol extract**	**Aqueous extract**
Carbohydrates	-	-	-	+
Alkaloids	-	-	+	-
Flavonoids	-	-	+	
Proteins and amino acids	-	+	-	-
Glycosides	+	-	+	-
Steroids	-	+	+	-
Terpenoids	-	-	-	-
Saponins		-	-	+
Tannins	-	+	+	-
Phenolic Compounds	-	+	+	-
Fats and oils	-	-	-	-

“+” Presence, “-” Absence

TOXICOLOGICAL STUDIES:

Acute Toxicity Study

There were no signs of toxicity or mortality, up to the limit dose of 2000 mg/kg in treated rats. All 24 rats were normal throughout the study and survived until the end of the 14-day experiment period. Animal wellness parameters were observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days as per paragraph 24 and 25 of OECD Guideline 423. Experimental observations are recorded systematically for each group. The parameters considered are changes in skin and fur, eyes and mucous membrane and also respiratory and circulatory, autonomic and central nervous system, somatomotor activity and behavioral pattern. Special attention is given for the observations of tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma.

Changes in wellness parameters observed for *Chromolaena Odorata* treated wistar rats.

Sl no	Response	Group1(5mg/kg)		Group 2 (50mg/kg)		Group 3 (300mg/kg)		Group4 (2000mg/kg)	
		Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Anxiety	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Roaming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Tremor	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Depression	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Scratching	Present	Present	Present	Present	Present	Present	Present	Present
10	Defecation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Pilo erection	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

18	Nail status	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Gauntness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
20	Gait	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
22	Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
23	Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	Lethargy	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
25	Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Subacute Toxicity Study:

Oral administration of ethanolic extract of *Chromolaena Odorata* root at doses of 200 and 500 mg/kg body weight daily for 28 days, did not produce any signs of toxicity or mortality. The animals did not show any changes in general behaviour or other physiological activities and were found normal throughout the study. The group 4 animals which were treated with 1000 mg/kg body weight of the extract showed mild signs of toxicity (Altered blood parameters). Little or no change was observed in body weight, food consumption, and water intake in extract (200, 500, and 1000 mg/kg) treated groups compared with control group after 28 days of study period in rats.

Table 8: Effect on body weight during the period of 28 days oral administration of ethanolic extract of *Chromolaena Odorata* root.

Group	0 th day	7 th day	14 th day	21 st day	28 th day
Group I (Control)	174.0±2.91	177.5±1.02	177.5±3.01	178.66±1.33	180.33±0.13
Group II (200 mg/kg)	172.0±2.07	172.0±1.28	176.66±2.13	179.13±4.34	181.67±3.57
Group III (500 mg/kg)	169.33±0.90	170.0±3.22	174.66±1.34	174.47±1.1	178.77±4.63
Group IV (1000 mg/kg)	171.0±3.11	175.33±0.18*	179.5±3.88**	181.83±4.04**	183.17±4.49**

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA). the values are statistically different from control at $p<0.01$**

Table 9: Effect on food intake and water intake during the period of 28 days of the oral administration of ethanolic extract of *Chromolaena Odorata* root.

Group	Item	0 th Day	7 th Day	14 th Day	21 st Day	28 th Day
Group I (Control)	Food	13.57±0.97	17.23±0.11	18.6±0.39	23.12±1.09	23.48±1.01
	Water	16.60±1.83	18.17±3.74	20.24±2.0	17.3±3.34	23.7±1.0
Group II (200 mg/kg)	Food	14.73±2.23	17.14±0.14	18.17±3.21	23.13±0.83	23.83±1.19
	Water	17.83±0.17	20.83±1.76*	23.83±3.12	23.33±0.18	24.16±1.48
Group III (500 mg/kg)	Food	17.70±2.16	19.06±3.22	21.42±3.30	23.05±2.07	25.09±4.01
	Water	21.04±2.84	24.17±4.67	26.9±1.73*	24.18±2.00*	25.0±1.03
Group IV (1000mg/kg)	Food	20.0±2.37*	19.67±2.78	22.67±3.17	22.4±1.44	26.17±3.81
	Water	19.65±0.74	18.04±2.30**	23.11±4.95	24.12±3.96**	24.73±2.55

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA). the values are statistically different from control at $p<0.05$*, $p<0.01$**

Haematological Studies

Haematological examination were done at the initial stage and at the end of the study. The blood samples were collected by retro orbital puncture. Hematological analysis is one of the important methods of assessing the toxicity of foreign compounds on blood constituents of an animal. Haematological parameters like Total Haemoglobin, Total WBC Count, differential leukocyte count, Total RBC Count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Platelet count were not significantly different with ethanolic extract of *Chromolaena Odorata* root treated rats from control group

Table 10: Effect on haematological parameters after 28 days of the oral administration of ethanolic extract of *Chromolaena Odorata* root.

Parameters	Sex	Day	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Hb	M	0	16.1±3.17	14.8±1.2	16.81±0.26	15.65±3.74
		28	16.4±2.74	15.71±2.08	14.67±1.98	15.69±1.67
	F	0	15.27±2.40	14.18±3.10	15.90±2.94	15.30±2.18
		28	16.31±1.18	14.18±0.47	15.10±1.18	14.20±2.14
RBC	M	0	9.14±1.03	8.78±1.40	9.17±1.88	9.87±0.12
		28	7.74±2.15	7.30±1.07	8.74±1.07	8.79±1.07
	F	0	8.47±0.47	9.70±1.03	8.79±1.19	9.04±1.07

		28	9.1±1.02	8.37±1.40	9.77±1.70	8.12±1.11
WBC	M	0	12.50±0.33	12.10±2.45	11.37±5.41	11.10±2.53
		28	10.40±0.74	10.50±1.70	11.0±1.70	13.14±0.22
	F	0	13.66±1.70	12.71±3.10	13.6±3.71	12.14±1.20
		28	13.17±3.60	13.07±3.76	12.98±2.77	12.63±0.53
Platelet Count	M	0	667.17±5.44	682.0±7.14	674.5±5.19	598.16±4.01
		28	614.7±6.09	620.27±4.17	675.3±6.08	602.16±8.71
	F	0	774.73±6.86	730.57±4.14	732.12±7.17	767.40±5.42
		28	731.33±10.56	734.37±6.81	783.32±5.01**	720.07±5.08**
MCV	M	0	56.32±0.10	59.23±1.43	56.72±0.22	58.25±2.52
		28	56.01±1.61	52.45±7.10	59.74±4.37	57.45±1.00
	F	0	61.01±1.50	60.90±4.90	61.70±3.12	60.71±5.92
		28	59.94±4.20	53.19±3.47	63.35±3.87	63.56±2.03
MCH	M	0	21.12±3.74	22.80±2.12	21.87±2.59	22.37±1.09
		28	22.15±3.40	21.03±3.17	22.07±3.41	21.96±1.92
	F	0	21.35±2.62	20.87±2.74	19.87±2.76	21.17±0.70
		28	22.04±3.12	21.74±2.78	21.79±1.61	20.87±3.49
MCHC	M	0	30.30±1.62	31.60±2.56	33.50±3.48	32.86±3.01
		28	33.01±2.07	30.31±2.32	31.04±2.05	31.87±2.27
	F	0+	31.09±3.83	31.70±4.05	31.10±3.83	32.87±2.27
		28	29.66±4.25	32.79±2.95	30.57±0.99	31.78±2.49
N	M	0	9.72±1.40	10.2±0.41	10.99±1.85	9.74±4.17
		28	10.37±3.08	11.2±3.60	12.34±4.07	11.9±2.39
	F	0	10.6±1.01	9.17±1.80	10.73±1.74	10.61±3.10
		28	11.93±2.41	10.03±3.44	11.9±4.01	16.13±1.69
L	M	0	65.17±1.60	78.83±3.30***	67.5±2.37	68.16±1.39
		28	70.03±1.68	79.50±1.52**	70.16±0.74	82.16±1.77***
	F	0	84.0±2.67	84.6±1.07	78.50±4.08	79.16±1.77
		28	81.53±1.37	80.07±1.73	80.67±0.81	82.5±1.99
E	M	0	2.09±0.47	1.76±0.17	1.66±0.88	1.66±0.33
		28	2.16±0.12	1.33±0.22	2.13±0.46	1.02±0.47
	F	0	1.60±0.61	1.03±0.55	2.33±0.33	1.66±0.66
		28	1.74±0.55	1.68±0.45	3.07±0.04	1.0±0.0
M	M	0	0	0	0	0

		28	0	0	0	0
	F	0	0.72±0.26	0.57±0.17	0.51±0.05	0.76±0.19
		28	0.64±0.17	0.63±0.18	0.83±0.16	1.05±0.21
B	M	0	0	0	0	0
		28	0	0	0	0
	F	0	0.4±0.48	0.2±0.10	0	0
		28	0.3±0.46	0.5±0.8	0.4±0.97	0

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA); the values are statistically different from control at p<0.05*, p<0.01** and p<0.001***.

Biochemical Analysis

Boichemical analysis should be performed on blood samples obtained of all animals to investigate major toxic effects in tissues and, specifically, effects on kidney and liver. The effects of the plant extract on biochemical parameters are summarized in the table below. Biochemical parameters like random blood glucose, aspartate transaminase (AST), alanine transaminase (ALT) and lipid profile did not show any difference with the above doses of ethanolic extract of *Chromolaena Odorata* root, compared to control group. Statistically there were significant variations were found in values of urea and creatinine of the animal group treated with 1000 mg/kg of extract, which is considered as a toxic sign. Detailed histopathological studies are to be planned in conforming the same.

Table 11: Effect on biochemical parameters after 28 days of oral administration of ethanolic extract of *Chromolaena Odorata* root.

Parameters	Sex	Day	Control	200mg/kg	500 mg/kg	1000 mg/kg
Random Glucose	M	0	89.10±2.13	87.06±1.78	86.63±1.97	90.30±1.42
		28	89.68±1.14	89.82±0.59	90.16±2.00	89.37±3.68
	F	0	85.21±1.33	89.87±1.01	86.65±0.62	89.28±2.05
		28	83.50±0.43	89.05±3.12	91.53±1.63	86.70±3.03
SGOT	M	0	31.52±2.07	32.05±1.6	31.32±1.04	31.54±1.50
		28	31.81±1.77	30.16±1.09	31.60±2.19	34.43±2.07
	F	0	33.74±2.75	32.03±2.87	32.91±0.81	35.41±3.07
		28	31.94±2.40	32.1±3.42	33.88±4.12	33.81±1.07
SGPT	M	0	35.12±1.76	35.78±1.21	34.10±1.08	35.6±1.29
		28	34.75±2.39	35.62±2.25	33.16±2.04	34.61±2.18
	F	0	34.82±2.50	33.87±2.56	34.15±2.84	35.07±1.37

		28	33.88±1.01	34.81±2.36	35.26±1.33	34.16±3.20
Urea	M	0	21.13±30.23	24.81±2.96	31.90±2.74	48.01±4.06*
		28	22.39±2.70	30.51±2.66	28.10±2.67	29.43±1.12
	F	0	26.15±1.40	28.43±2.68	29.74±1.73	41.45±1.68*
		28	27.17±2.21	31.04±1.65	31.63±3.26	31.07±2.36
Creatinin	M	0	0.32±0.13	0.44±0.08	0.41±0.06	0.47±0.44
		28	0.36±0.08	0.37±0.04	0.37±0.06	1.42±0.03
	F	0	0.41±0.12	0.36±0.14	0.44±0.27	0.49±0.17
		28	0.46±0.27	0.36±0.16	0.48±0.63	1.44±0.16

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA); the values are statistically different from control at p<0.05*, p<0.01** and p<0.001***.

Lipid Profile

Lipid profile parameters like Total cholesterole ,LDL, VLDL, HDL and Triglyceride were found to be normal for ethanolic extract of *Chromolaena Odorata* root treated rats when compared with control groups. The study shows that there are no significant changes observed in the lipid profile values when compared with control group. ethanolic extract of *Chromolaena Odorata* root at 1000 mg/kg shows significant reduction in triglycerides indicative of anti-hyperlipidaemic activity.

Table 12: Effect on Lipid profile after 28 days of oral administration of ethanolic extract of *Chromolaena Odorata* root.

Parameters	Sex	Day	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Total Cholesterol	M	0	94.66±1.68	93.77±2.02	91.42±1.17	89.16±3.19
		28	97.71±2.16	92.30±2.30	89.10±1.06	91.53±3.25
	F	0	101.71±2.81	96.13±3.12	93.90±1.46	95.24±3.05
		28	98.90±2.65	94.73±2.02	93.47±3.17	90.19±2.40
Triglycerides	M	0	88.03±3.02	100.10±2.81	96.57±1.18	89.17±4.06
		28	96.13±3.71	102.03±2.90	98.77±2.74	69.60±1.53***
	F	0	107.90±3.37	107.57±2.35	99.16±2.78	89.71±1.25
		28	98.03±4.41	103.91±2.43	98.90±1.12	71.87±1.86***
HDL	M	0	44.17±5.42	42.81±3.08	46.17±3.16	40.87±2.76
		28	43.91±4.27	42.33±2.31	46.76±3.88	41.59±2.03
	F	0	44.12±2.76	42.02±2.50	43.02±3.09	40.14±2.96
		28	46.47±2.15	45.14±1.63	44.70±2.31	37.29±2.18

LDL	M	0	36.10±2.13	34.30±1.03	32.41±2.16	28.28±2.02
		28	34.31±4.77	33.57±9.61	32.67±2.79	30.44±1.02
	F	0	32.80±3.20	34.97±2.98	34.19±3.15	32.84±2.94
		28	41.34±3.19	38.70±1.60	35.91±4.49	30.28±3.19**
VLDL	M	0	20.61±1.23	21.36±3.30	20.18±2.75	18.40±1.83
		28	23.62±3.87	22.36±2.99	20.16±1.36	18.47±3.04
	F	0+	23.47±3.17	25.24±4.56	21.07±3.29	20.74±3.18
		28	24.47±4.99	25.32±3.18	20.55±3.67	18.03±3.94

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA); the values are statistically different from control at p<0.05*, p<0.01** and p<0.001***.

Organs Weight and Histology

The organs like kidney, heart, and brain isolated in various groups did not show any abnormalities in their gross examinations and difference in their mean weights both in treated and control groups exept with the kidney and liver of 1000mg/kg extract treated group. Histopathological study slides and reports shows that claims that all other extract treated groups showed normal histological architecture.

Table 13: Effect on isolated organs weight after 28 days of the oral administration of ethanolic extract of *Chromolaena Odorata* root.

Group	Brain	Heart	Kidney	Liver
Group I (Control)	1.26±1.34	1.03±1.0	1.07±0.68	4.91±0.75
Group II (200 mg/kg)	1.32±0.14	0.94±0.17	1.08±0.36	4.11±0.68
Group III (500 mg/kg)	1.36±1.16	1.01±0.29	1.21±0.48	4.32±0.34
Group IV (1000mg/kg)	1.32±0.31	0.98±1.31	1.92±1.09*	5.96±0.29*

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA). Values are not statistically different.

Histopathological Study Results.

The histological study of the liver of group IV showed that the lobular architecture was maintained; also individual hepatocytes show microvesicular steatosis. The central vein shows congestion. The portal triad shows bile duct hyperplasia. The sinusoids were dilated. Histopthological results of all other groups showed normal histological arichitecture.

HITOPATHOLOGY IMAGES

Image 7: Histology of brain from control and extract treated rats.

Histology of Brain of control group and extract treated group. Brain section from control group (fig.1) shows normal cerebellum with brain parenchyma shows normal morphology, no evidence of neuronal degeneration. Fig. 2, 3 and 4 –Brain cells from extarct 200, 500 and 1000 mg/kg group also exhibits normal cerebellum with brain parenchyma shows normal morphology, no evidence of neuronal degeneration.

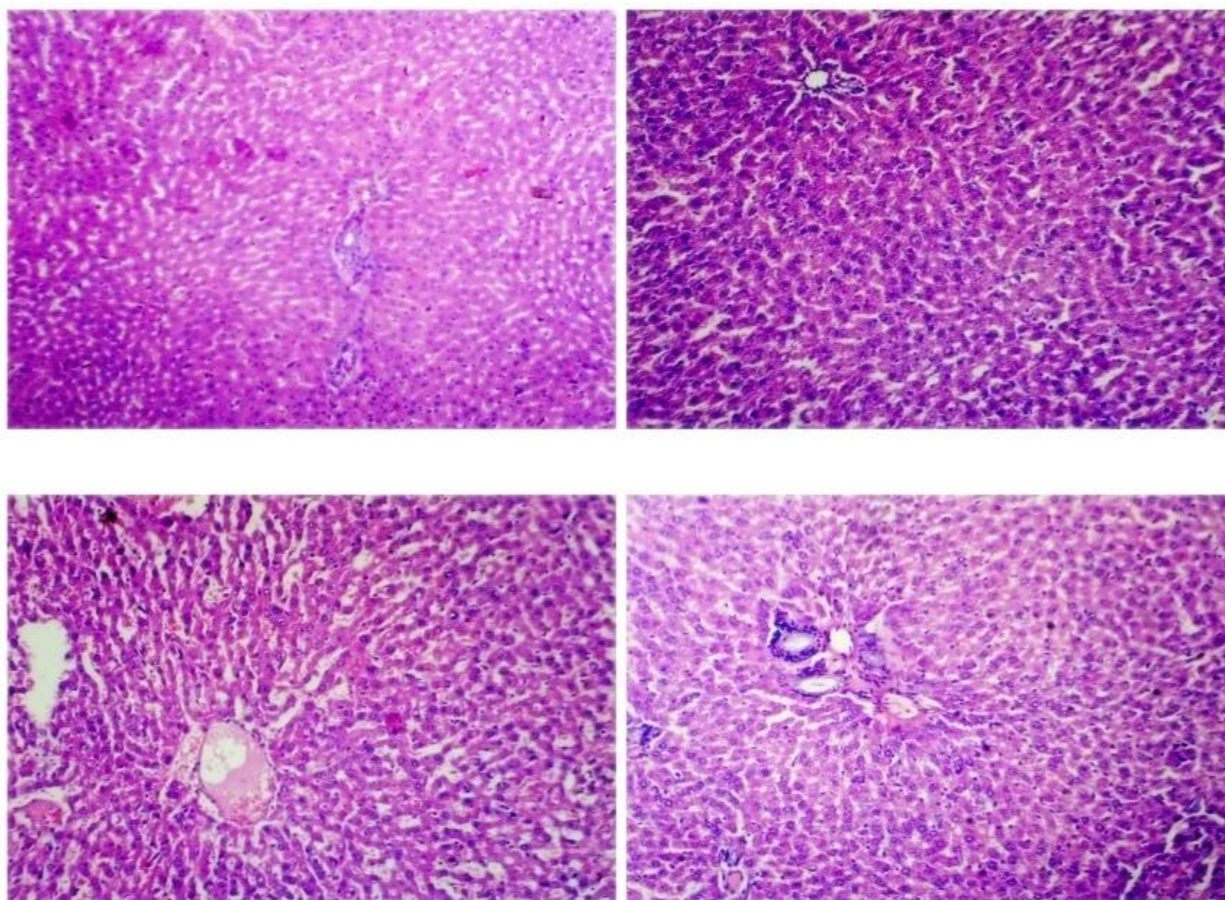


Image 8: Histology of liver from control and extract treated rats.

Histology of liver of control and extract treated animals. Section of liver from control animals (fig 5) revealed normal lobular architecture and there is no evidence of binucleation cytoplasmic vacuolation or inflammation; the hepatocytes of 200 and 500 mg/kg (fig 6 and 7) also show normal lobular architecture and there is no evidence of binucleation cytoplasmic vacuolation or

inflammation. 1000 mg/kg (fig 8) showed normal lobular architecture but individual hepatocytes show micro vesicular steatosis. The central vein show congestion and the portal triad shows bile duct hyperplasia. The sinusoids are dilated.

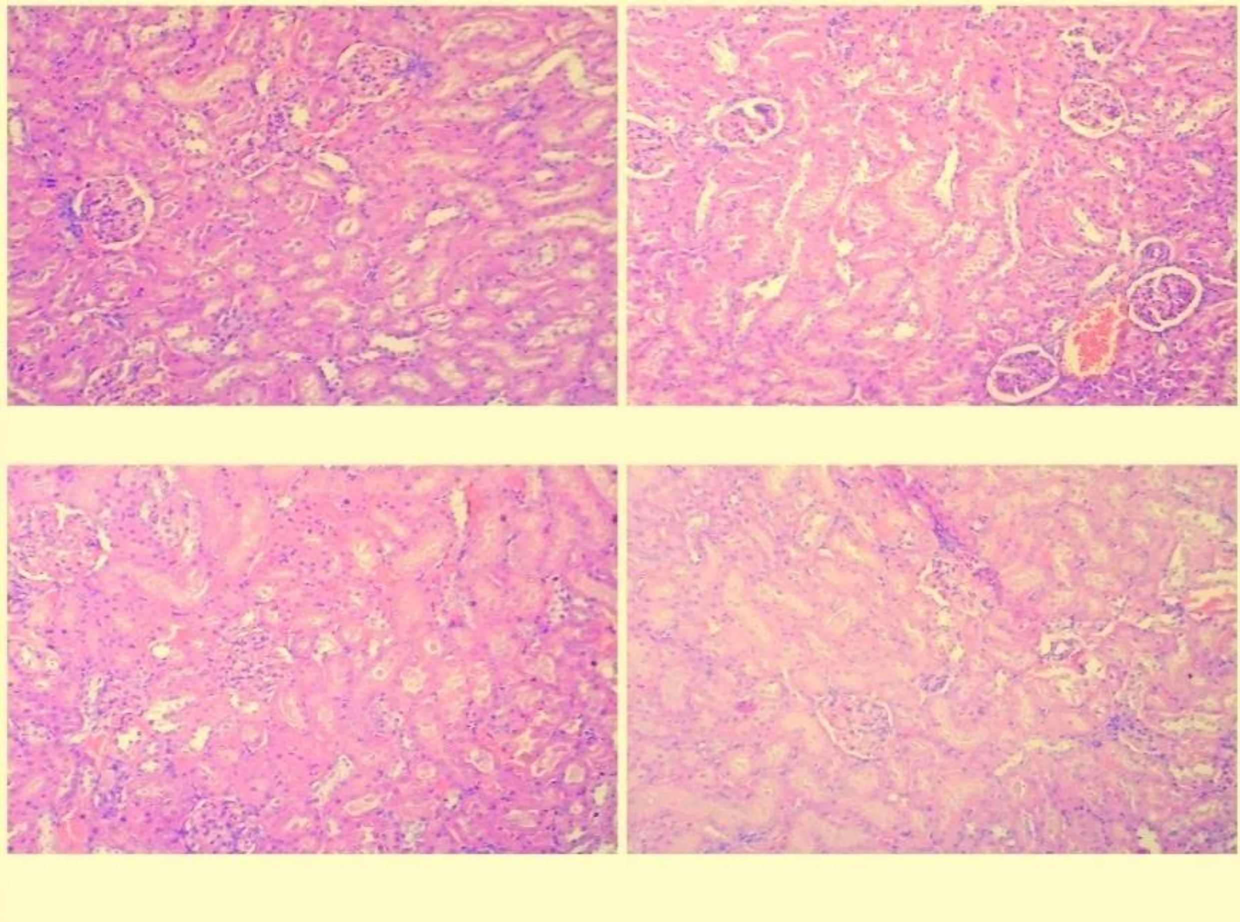


Image 9: Histology of kidney from control and extract treated rats.

Histology of kidney of control and extract treated animals. Section of kidney from control animal (fig 9) shows normal medulla, cortex and glomeruli. The tubulointerstitial compartments shows unremarkable. The collecting duct of the medulla also shows normal in morphology. There is no evidence of inflammation or necrosis; renal histology of extarct treated groups (fig 10,11 and 12) are also found to be same as control group.

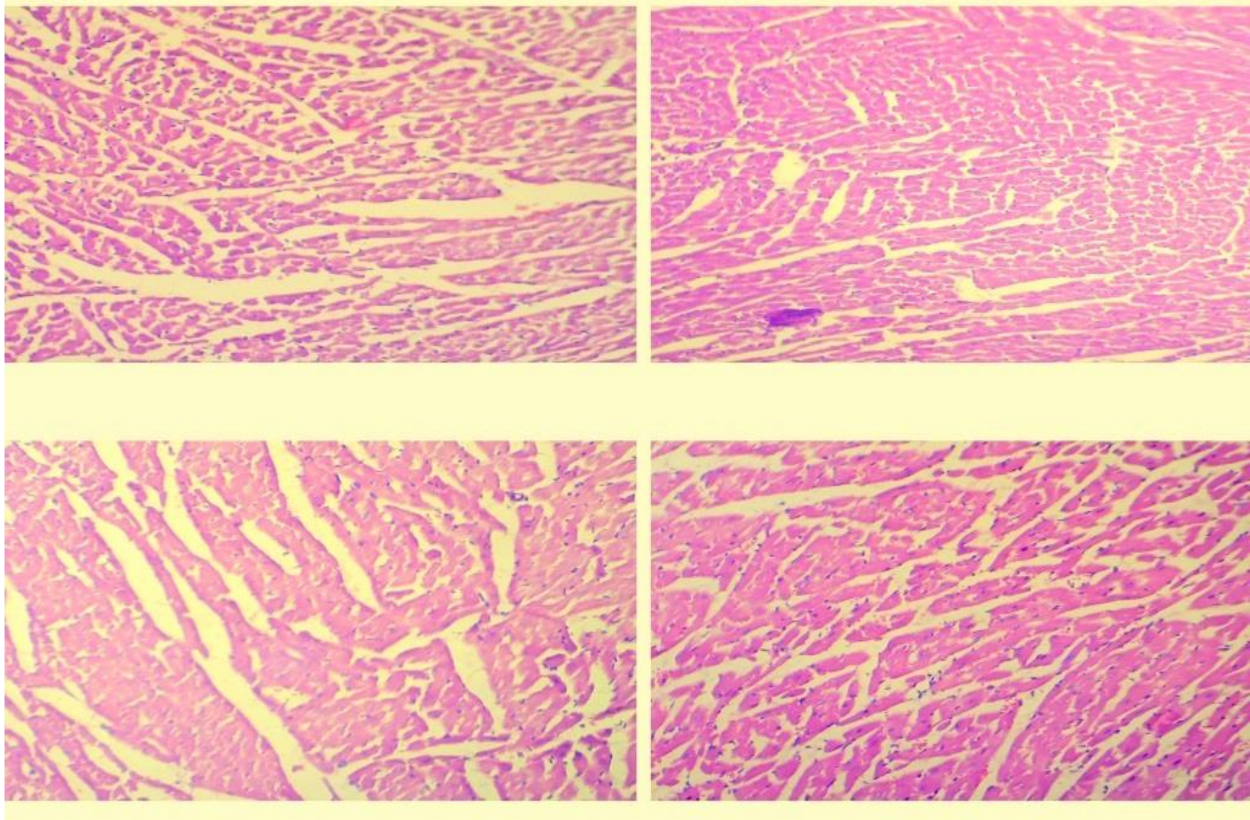


Image 10: Histology of heart from control and extarct treated rats.

Histology of heart of control and extarct treated animals. Section of heart from control animal (fig 13) showed normal myocardium with myocytes. The blood vessels are unremarkable. There is no evidence of myocytic degeneration or edema or inflammation; the extarct treated rats (fig 14, 15 and 16) also exhibits the same microscopy

PHARMACOLOGICAL SCREENING FOR ANALGESIC ACTIVITY

TAIL FLICK METHOD

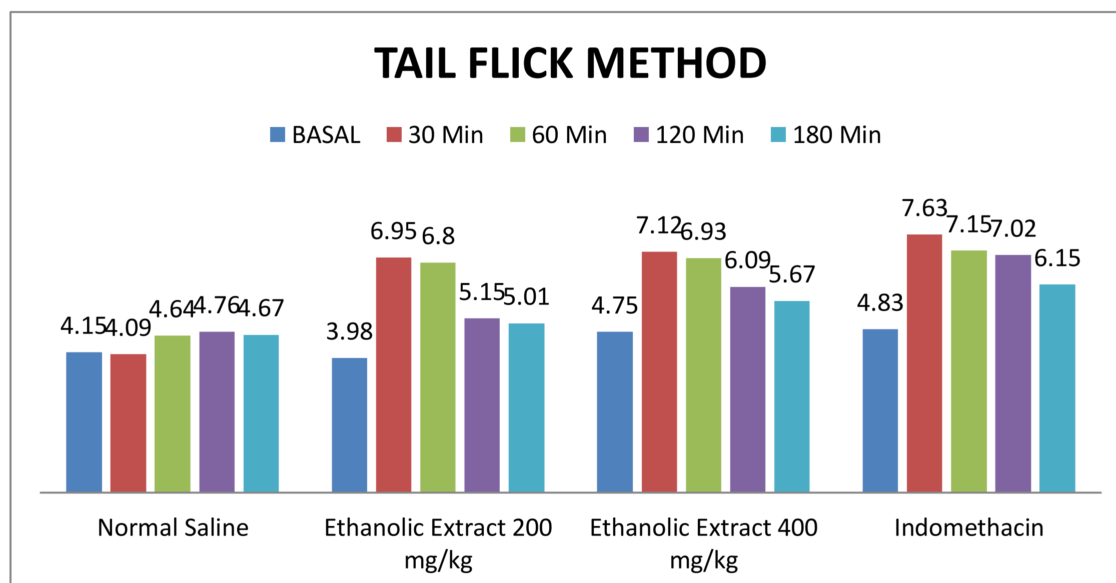
Table-14 Effect of Analgesic Activity of the ethanolic extract of *Chromolaena Odorata* roots by using tail flick method.

All values are given in mean±SD, (n=6) ANOVA **p<0.05, *p< 0.01 when compared to Control

Treatment	Dose/Route	**Reaction time in sec (mean ± SEM)**				
		Basal	30 min	60min	120min	180min
Normal saline	10ml/kg/p.o	4.15±0.013	4.09±0.012	4.64±0.025	4.76±0.017	4.67±0.22
Ethanolic extract of *Chromolaena Odorata*	200mg/kg/p.o	3.98±0.041	6.95±0.032**	6.80±0.021**	5.15±0.29	5.01±0.11
Ethanolic extract of *Chromolaena Odorata*	400mg/kg/p.o	4.75±0.012	7.12±0.023**	6.93±0.045**	6.09±0.022	5.67±0.001
Indomethacin	5mg/kg/p.o	4.83±0.019	7.63±0.017**	7.15±0.027**	7.02±0.03**	6.15±0.018

group

Fig 11: Effect of analgesic activity of ethanolic extract of *Chromolaena Odorata* by using tail flick method.



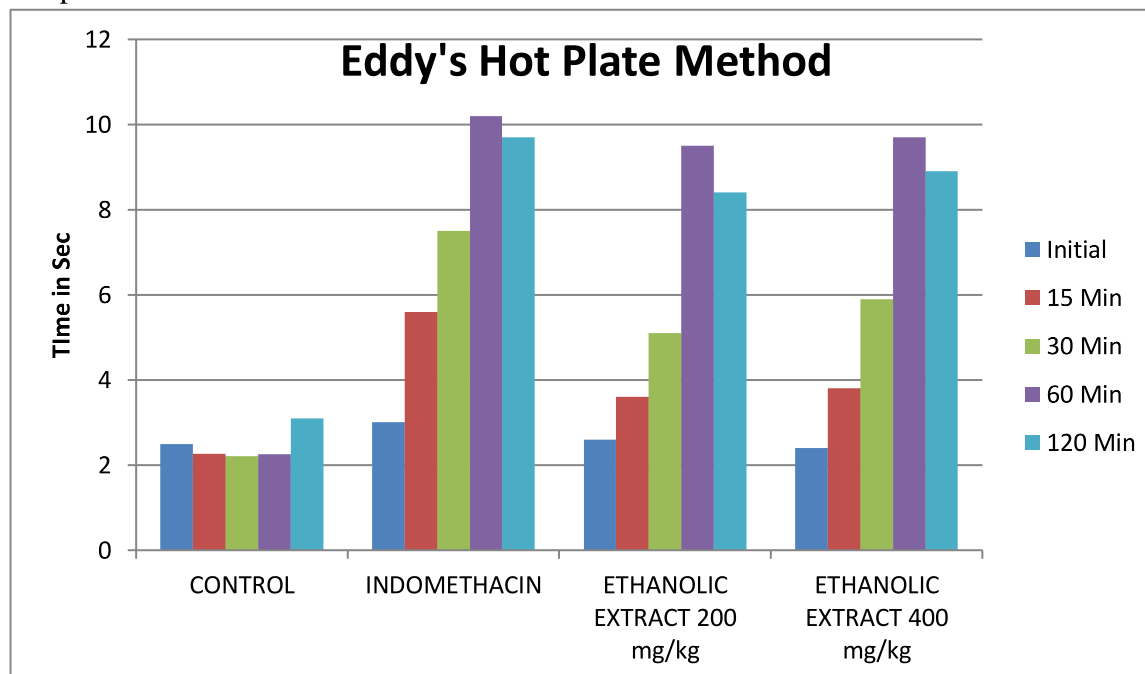
EDDY'S HOT PLATE METHOD

Table-15 Effect of analgesic activity of ethanolic extract of *Chromolaena Odorata* by Eddy's hot plate method

Treatment	Dose	Reaction time in Sec (Mean±SEM)				
		0 min	15min	30min	60min	120min
Control	10ml/kg	2.5±0.3	2.27±0.1	2.21±0.5	2.25±0.6	3.1±0.4
Indomethacin	5mg/kg	3.0±0.2	5.6±0.4	7.5±0.3**	10.2±0.2**	9.7.±0.3
Ethanolic extract of *Chromolaena Odorata*	200 mg/kg	2.6±0.2	3.6±0.5	5.1±0.6	9.5±0.4**	8.4±0.1
Ethanolic extract of *Chromolaena Odorata*	400 mg/kg	2.4±0.3	3.8±0.3	5.9±0.2	9.7±0.3**	8.9±0.1

All values are given in mean±SD, (n=6) ANOVA **p<0.05, *p< 0.01 when compared to control group

FIG- 12: Effect of analgesic activity of ethanolic extract of ***Chromolaena Odorata*** by Eddy's hot plate method.



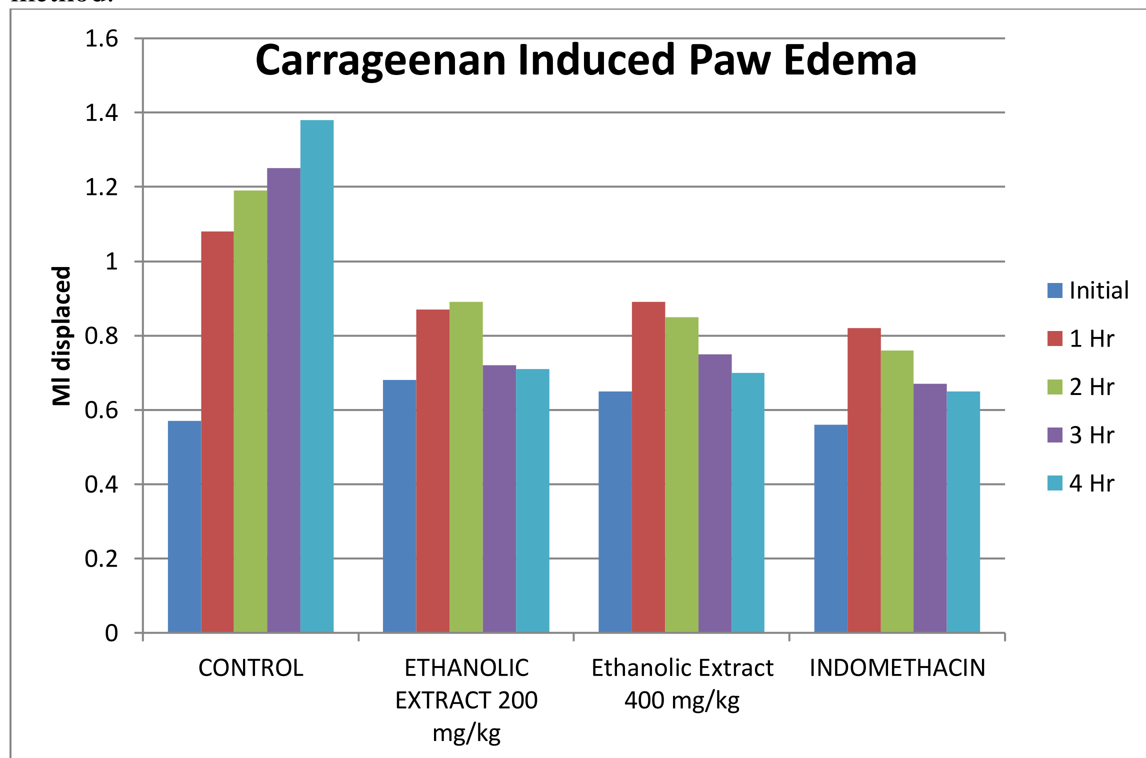
ANTIINFLAMATORY ACTIVITY

Table-16 Effect of Anti-inflammatory activity of ethanolic extract of *Chromolaena Odorata* by Carrageenan induced paw edema method.

Treatment	Dose	Mean Paw volume(ml)				
		Before	+1 hr	+2hr	+3hr	+4hr
Control		0.57±0.21	1.08±0.16	1.19±0.16	1.25±0.13	1.38±0.2
Ethanolic extract of *Chromolaena Odorata*	200mg/kg/p.o	0.68±0.26	0.87±0.15	0.89±0.20*	0.72±0.3**	0.71±0.2
	400mg/kg/p.o	0.65±0.11	0.89±0.15	0.85±0.21*	0.75±0.19**	0.70±0.5
Indomethacin	5mg/kg/p.o	0.56±0.15	0.82±0.18	0.76±0.23**	0.67±0.21**	0.65±0.7

All values are given in mean±SD, (n=6) ANOVA **p<0.05, *p< 0.01 when compared to control

Fig – 13: Effect of Anti inflammatory activity of BCLE by Carrageenan induced paw edema method.



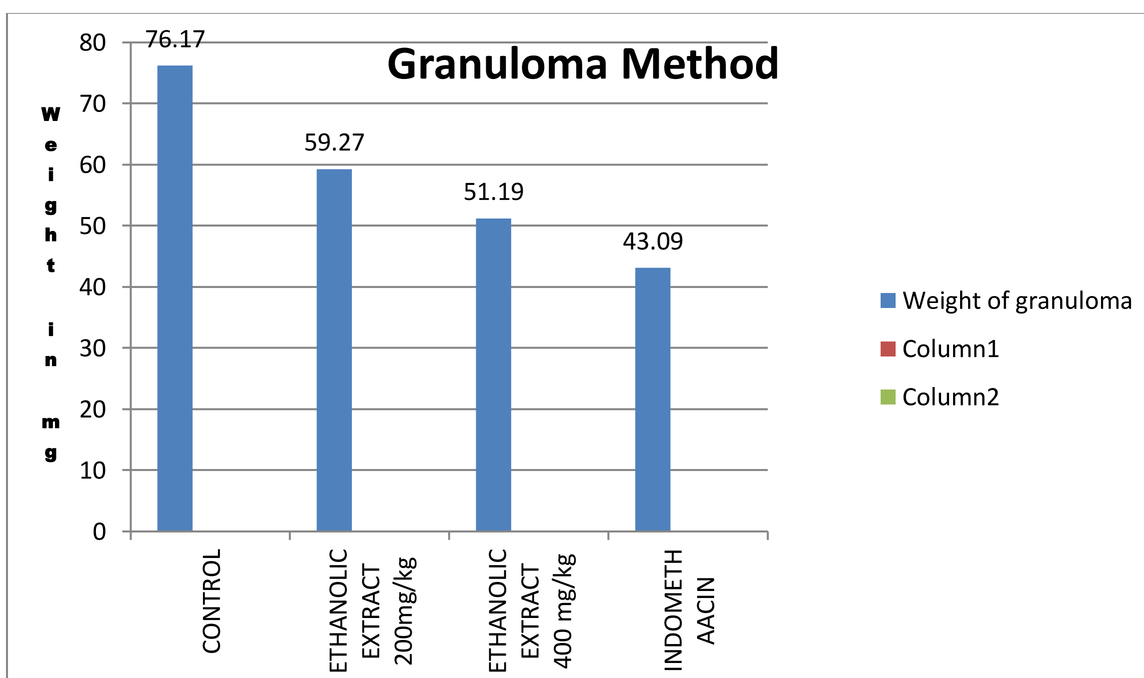
GRANULOMA METHOD

Table-17 Effect of Anti-inflammatory activity of ethanolic extract of *Chromolaena Odorata* by cotton pellet induced grauloma method

DRUGS	DOSAL ROUTE	WEIGHT OF DRY COTTON PELLET OF GRANULOMA MG(Mean±SEM)
Control	-	76.17±0.37
Ethanolic extract of *Chromolaena Odorata*	200mg/kg	59.27±0.25*
Ethanolic extract of *Chromolaena Odorata*	400mg/kg	51.19±0.17**
Indomethacin	5mg/kg	43.09±0.24**

All values are given in mean±SD, (n=6) ANOVA **p<0.05, *p< 0.01 when compared to control group.

Fig -14: Effect of Anti-inflammatory activity of ethanolic extract of ***Chromolaena Odorata*** by Cotton Pellet induced granuloma method



DISCUSSION

DISCUSSION

Chromolaena odorata is a scrambling perennial shrub which grows 2–3 m in height with straight, pithy, brittle stems that branch readily. The plant genus *Chromolaena* is a genus of the family *Asteraceae* which comprises over 165 species that are distributed across tropical and subtropical regions. *C. odorata* is often noted as a native of tropical Central and South America, from Mexico and the Caribbean to Brazil; however, the exact northern and southern limits of its native range remain uncertain and are likely to also include some regions outside of the tropics. Plant as a whole contains the following phytoconstituents like; flavonoid aglycones (flavanones, flavonols, flavones) including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin; terpenes and terpenoids; essential oils; alkaloids including pyrrolizidine, saponins and tannins; phenolic acids (including ferulic acid, protocatechuic acid); phytoprostane compound including chromomoric acid.

In the current project, root was selected as the sample for study. The study started with evaluating the best extract out of the different solvent extraction technique. The phytochemical evaluation and extractive value were the strategies fixed to choose the best extract from a list of five different extracts wiz petroleum ether, chloroform, ethanol, acetone, water. The ethanolic extract showed superior range of phytoconstituents like alkaloids, flavonoids, glycosides, steroids, tanins, phenolic compounds and maximum extractive value (4.03). The active phytochemical constituents may be linked to the analgesic and anti-inflammatory activity of the plant.

To derive the safety profile of the roots of *Chromolaena odorata,* it was subjected to the acute toxicity study which conformed the absence of any toxicity or mortality at the higher dose of 2000mg/kg. Thus the extract 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 200mg/kg is used as a dose of extract and middle dose 500mg/kg(Dose×2.5) and higher dose as 1000mg/kg (Dose×5) were considered for further sub-acute toxicity studies.

Further 28 days continues and detailed toxicological evaluation was performed. Sub acute toxicity study revealed that the extract is safe upto 500 mg/kg dose for the continues

administration. Also, all the animals from control and all the treated groups up to dose 500 mg/kg survived throughout the dosing period of 28 days. Animals from all these treated groups exhibited comparable body weight gain with that of controls throughout the dosing period. Significant changes in the organ weight and biochemical parameters were observed to the animal groups treated with 1000 mg/kg of test extract. Specific cellular damages were noticed by the microscopic examination during the histopathological evaluation of group V animals (1000 mg/kg). The central and autonomic profiles were normal throughout the study and no specific alterations were noticed. The results of the effect of the extract on the body weight of the animals compared with vehicle are as shown in Table. There were no significant increases in the weight of animals treated with 200 mg BCLE. The results of the effect of BCLE on absolute organ weights of male and female rats are as shown in Table . Macroscopic examination did not show any changes in the colour of organs of the treated animals compared with vehicle. There were significant changes in the relative weight of the liver and kidney in both males and females. Treatment had no effect on spleen stomach and testes of male rats. Hematological analysis and Biochemical analysis conducted at the end of the dosing period revealed no abnormalities attributable to the treatment till the dose of 500 mg/kg of extract.

Carrageenan induced paw odema is the most widely used screening method for anti-inflammatory drugs. Screening of anti-inflammatory activity of ethanolic extract of *Chromolaena odorata* was done by Carrageenan induced paw odema and by Granuloma method in rat model. While screening of analgesic activity is done by using Eddys hot plate in swiss albino mice model and Tail flick method by Wistar rat model. The ethanolic extracts of *Chromolaena odorata* in varying doses such as (200 and 400mg/kg body) was given orally. From the table showed that methaolic extracts of *Chromolaena odorata* showed significant reduction in the paw odema at 3 hr and is quite similar when compared to the group treated with standard drug indomethacin (5mg/kg). 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 Bradykinin, leukotrienes, Polymorphonuclear cells. The highest percentage of inhibition was found with the dose of 400mg/kg. In case of Granuloma method the ethanolic extracts of Bombax Ceiba showed significant activity. The maximum inhibition of granuloma occurs at 400mg /kg dosing which is quite similar to the group treated with standard drug indmethacin (5mg/kg).

Granuloma formation occurs in diseases like tuberculosis leporosy etc. Granuloma method [91-93] involves three phases. Initial phase transudative phase occurs during the first 3 hours secondly an exudative phase occurs between 3 and 72 hours after the implantation of the pellet. Then a proliferative phase measured as increased in dry weight of the granuloma that occurs between 3 and 6 days after implantation. The dry weight of granuloma was decreased by ethanolic extracts of *Chromolaena odorata* 400mg/kg. The antiproliferative effect of *Chromolaena odorata* is was comparative to that of the standard drug administered. Eddys hot plate method and Tail flick test are used for the evaluation of the central pain at the supra spinal and spinal levels. Tail flick model is highly correlative with relief of human pain perception. Tail flick response is believed to be spinally mediated reflex and paw licking response is complex supraspinally orginated reflex. In case of Eddy's hot plate and Tail flick methods the ethanolic extract of *Chromolaena odorata* (200mg/kg,400mg/kg) increase the stress tolerance capacity of animals. In Tail flick test the time taken to reach the peak analgesic activity +30 and +60 minute 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 *Chromolaena odorata* possess significant analgesic and anti-inflammatory activity.

CONCLUSION

CONCLUSION

The presented study was an attempt to investigate the Analgesic and Anti-inflammatory activity of extract of *Chromolaena odorata* roots.

The Phytochemical screening showed the presence of Alkaloids, Flavonoids, Glycosides, Steroids, Tanninns and Phenolic compounds which is expected to be responsible for Analgesic and Anti-inflammatory activity.

Detailed assessment in the extractive values and the phytochemical analysis revealed that ethanolic extract of *Chromolaena odorata is the best extract to be evaluated for the activity.*

The finding of the present investigation suggests that the ethanolic extract of *Chromolaena odorata* has potential Analgesic and Anti-inflammatory activity.

Toxicity studies do not produce any toxic symptoms on Acute evaluation and during Sub acute toxicity studies the animal groups administered with 1000 mg/kg of the extract showed signs of toxicity.

In conclusion the ethanolic extract of *Chromolaena odorata* are safe upto the dose of 500 mg/kg for continues administration and can be used as Analgesic and Anti-inflammatory agent without any harmful effects.

Further studies are required to confirm the exact mechanism behind the Analgesic and Anti-inflammatory activity of *Chromolaena odorata* ethanolic leaf extract.

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