

**EVALUATION OF JUSTICIA GEND ARUSSA LEAVES EXTRACT
FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY****Jayshree. R Aate*¹ and Dr. Chandrashekhar. Tenpe²**¹Assistant Professor at Hi-Tech College of Pharmacy Chandrapur, Maharashtra²Associate Professor at Institute of Pharmaceutical Education and Research, Wardha,
Maharashtra.Article Received on
07 Nov. 2015,Revised on 28 Nov. 2015,
Accepted on 18 Dec. 2015,***Correspondence for
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tech college of pharmacy
chandrapur, Maharashtra.**ABSTRACT**

The Aim of The Present Study Was To Evaluate The Anti-Inflammatory And Analgesic Activity of The Different Extract of Leaves Justicia Gendarussa (EJG) In Animal Models. The Anti-Inflammatory Activity of The Extract Was Evaluated By Using Carrageenan-Induced Rat Paw Edema Method. The Analgesic Activity of The Evaluated For Its Central and peripheral pharmacological actions by using eddy's hot plate method. The study was carried out in two different dose levels of 150 and 300 mg kg⁻¹ orally. the EJG did not produce any mortality up to 2000 mg kg⁻¹ the ejg at the dose 300 mg kg⁻¹ showed maximum inhibition of 50% in carrageenan

induced paw edema. Dose dependent increase in latency of response in the hot plate method were observed with ejg at the dose 300 mg kg⁻¹. the pharmacological screening of the extract showed significant (p<0.001-0.01) dose – dependent Anti-Inflammatory Activity with good Analgesic profile when compared with reference standard. The presence of flavonoids might be responsible for these activities and which are probably mediated via inhibition of various autocooids pormation release.

KEYWORDS: Justicia Gendarussa, peripheral, autocooids pormation.**INTRODUCTION**

The defense mechanism of living tissue is some time pathophysiologically manifested as inflammation. Drug which are used presently for the management of pain and inflammatory conditions are either narcotics e.g opoids or non-norcotics e.g salicylates and corticosteroids. All of these drugs have well documented toxic effect. Prolonged use of both steroidal and

non-steroidal anti-inflammatory drugs is well known to be associated with peptic ulcer formation. Hence search for new anti-inflammatory agents that possess therapeutic efficacy and yet are devoid of these adverse effects is justified.

Now day's herbal drugs are being proved as effective as synthetic drug with lesser side effects. For last several years screening of indigenous medicinal plants is done to develop safe and potent anti-inflammatory drug for clinical use.^[1]

Herbal medicines are derived from plant extract are being increasing utilized to treat a wide range of clinical diseases, through relatively little knowledge about their mode of action is available. There is growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. e.g *Vitex negundo*, *Erigeron floribundus* and *Gymnema sylvestre*.

Inflammation is a universal host defense process involving a complex network of cell-cell, cell-mediator and tissue interactions. It occurs in response to a variety of stimuli viz. Physical, chemical, traumatic, antigen challenge and infectious agents. Apart from exogenous factors (physical, chemical, mechanical, nutritional and biological etc.), endogenous factors (immunological reaction, neurological and genetic disorders) also contribute to inflammatory response. Inflammation most commonly occurs when microbial invasion or tissue injury overcomes the body's nonspecific defense mechanisms. Subsequent to infection, immune system gets activated, communication and coordination occurs between different classes as well as actions of immune cell to produce inflammation.

There are three principle components of an inflammatory response: (i) increased blood flow, (ii) increased capillary permeability and (iii) increased migration of leucocytes into the affected area. Active hyperemia, exudation and accumulation of neutrophils and macrophages are observed at the inflammatory site in an inflammatory response.

SIGN OF INFLAMMATION

The Roman writer Celsus in 1st century A.D. named the famous 4 cardinal signs of inflammation as.

Rubor (redness); Tumor (swelling); Calor (heat); and Dolor (pain).

TYPES OF INFLAMMATION: Depending upon the defense capacity of the host and duration of response, inflammation can be classified as

I. Acute inflammation**II. Chronic inflammation****I. Acute inflammation**

It can be initiated by either immunological or non-immunological stimuli. During acute inflammation there will be a brief vasoconstriction of the arteriolar vessels followed by vasodilatation. The endothelial cells contract and plasma proteins leak out in proportions inversely related to their molecular sizes.

In 1957, Spector and Willoughby showed clearly that histamine is the first substance to be released, presumably from adjacent tissue mast cells. This is followed by 5HT and kinin and then, eicosanoids. The initial moment of these soluble mediators is followed by migration of polymorph nuclear leucocytes (PMNs) in response to chemo attractants like G5a along with G3a.^[9]

The compliment system is also activated during bacterial infection and immune mediated acute inflammation in which antigen combine with antibody. Leukotriene B4 (LTB4) is also a powerful chemo attractant, Which promotes the migration of polymorph nuclear leucocytes.

The emigrating cells remove both the damaged tissue and the initiating inflammatory stimulus by a process called phagocytosis under normal condition. The monocytes which migrate with the polymorphs in case of acute inflammation are capable of removing the debris, which is not for PMNs. The monocytes during its migration from blood vessels to the site of inflammation changes to macrophages, a cell that is ten times more phagocytic than PMNs. These two types of cells in combination with vascular changes are able to resolve the acute inflammation, which then slowly regresses and turns normal.

The changes in acute inflammation can be conveniently described under the following 2 heading

I. Vascular events.

II. Cellular events.

I. VASCULAR EVENTS

Alteration in the microvascular (arterioles, capillaries and venules) is the earlist response to tissue injury. These alteration include: haemodynamic changes and changes in vascular permeability.

Haemodynamic Changes

The earliest features of inflammation response results from changes in the vascular flow and caliber of small blood vessels in the injured tissue. The sequence of these changes is as under:

1. Irrespective of the type of injury, immediate vascular response is of transient vasoconstriction of arterioles. With mild form of injury, the blood flow may be re-established in 3-5 seconds while with more severe injury the vasoconstriction may last for about 5 minutes.
2. Next follows persistent progressive vasodilation which involves mainly the arterioles, but to a lesser extent, affects other components of the microcirculation like venules and capillaries. This change is obvious within half an hour of injury. Vasodilation results in increased blood volume in microvascular bed of the area, which is responsible for redness and warmth at the site of acute inflammation.
3. Progressive vasodilation, in turn, may elevate the local hydrostatic pressure resulting in transudation of fluid into the extracellular space. This is responsible for swelling at the local site of acute inflammation.
4. Slowing or stasis of microcirculation occurs next. Slowing is attributed to increased permeability of microvascular that results in increased concentration of red cells, and thus, raised blood viscosity.
5. Stasis or slowing is followed by leucocytic margination or peripheral orientation of leucocytic along the vascular endothelium. The leucocyte stick to the vascular endothelium briefly, and then move and migrate through the gaps between the endothelial cells into the extravascular space. This process is known as emigration.

MECHANISUM OF INCREASED VASCULAR PERMEABILITY

In acute inflammation, normally non-permeable endothelial layer of microvasculature become leaky. This explained by one or more of the following mechanisms

- i) **Contraction of endothelial cells.** This is tne most common mechanism of increased leakiness that affects venules exclusively while capillaries and arterioles remain unaffected. The endothelial cells develop temporary gaps between them due to their contraction resulting in vascular leakiness. It is mediated by the release of histamine, bradykinin and other chemical mediators.

ii) Retraction of endothelial cells. In this mechanism, there is structural re-organisation of the cytoskeleton of endothelial cells that causes reversible retraction at the intercellular junctions. This change too affects venules and is mediated by cytokines such as interleukin-1 and tumour necrosis factor.

iii) Direct injury to endothelial cells. Direct injury to endothelium causes cell necrosis appearance of physical gaps at the sites of detached endothelial cells. Process of thrombosis is initiated at the site of damaged endothelial cells. The change affect all levels of microvasculature.

iv) Endothelial injury mediated by leucocytes. Adherence of leucocytes to the endothelium at the site of inflammation may result in activation of leucocytes. The activated leucocytes release proteolytic enzymes and toxic oxygen species which may cause endothelial injury and increase vascular leakiness.

v) Neovascularisation. In addition, the newly formed capillaries under the influence of vascular endothelial growth factor during the process of repair and in tumours are excessively leaky.

II. CELLULAR EVENTS

The cellular phase of inflammation consists of 2 processes

1. Exudation of leucocytes
2. phagocytosis

Exudation of leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammation response. In acute inflammation, polymorph nuclear neutrophils (PMNs) comprise the first line of body defence, followed later by monocytes and macrophages.

The changes leading to migration of leucocytes are as follows

1.CHANGES IN THE FORMED ELEMENTS OF BLOOD. In the early stage of inflammation, the rate of flow of blood is increased due to vasodilation. But subsequently, there is slowing or stasis of bloodstream. With stasis, changes in the normal axial flow of blood in the microvasculature take place. The normal axial flow consists of central stream of

cells comprised by leucocytes and RBCs and peripheral cell- free layer of plasma close to vessel wall.

2.ROLLING AND ADHESION. Peripherally margined and paved neutrophils slowly roll over the endothelial cells lining the vessel wall.This is followed by the transient bond between the leucocytes and endothelial cells becoming firmer.

3.EMIGRATION. After sticking of neutrophils to endothelium, the former move along the endothelial surface till a suitable site between the endothelial cells is found where the neutrophils throw out cytoplasmic pseudopods. Subsequently, the neutrophils lodged between the endothelial cells and basement membrane cross the basement membrane by damaging it locally with secreted collagenases and escape out into the extravascular space; this is known as emigration.

4.CHEMOTAXIS. The chemotactic factor- mediated transmigration of leucocytes after crossing several barriers (endothelial, basement membrane, perivascular myofibroblasts and matrix) to reach the interstitial tissues is called chemotaxis.

Phagocytosis: Phagocytosis is defined as the process of engulfment of solid particulate material by the cells. The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells

- i) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response, also called as microphages.
- ii) Circulating monocytes and fixed tissue mononuclear phagocytes called as macrophages.

The process of phagocytosis is similar for both polymorphs and macrophages and involves the following 4 steps

- 1.Recognition and attachment stage
- 2.Engulfment stage
- 3.Secretion stage
- 4.Digestion or degradation stage.

II. CHRONIC INFLAMMATION

It is an extension of acute inflammation. There are three possible mechanisms for the transformation of inflammation from the acute to chronic stages.

The mechanisms are

1. Persistence of the initial inflammation stimulus. During this time there is a greater monocytes migration from the blood to the inflammatory site, with considerable intracellular interaction between the monocyte macrophage population and lymphocyte of T series.
2. The hydrolytic enzymes, released by cells during phagocytosis, are capable of degrading protein. Incomplete degradation of such protein results in an immunogenic product that is persistent and capable of acting as a stimulus for chronic inflammatory response.
3. The regulatory substance which are secreted by the cells during normal process of removal of debris in case of acute inflammation become aberrant in some way.

Interleukin 1 (IL-1) produced by macrophages activates T lymphocytes with interleukin 2 (IL-2). This helps the differentiation of T cells into various functional subsets such as T helper, T suppressor, and T cytotoxic cells. It is possible that certain other cytokines controls the type of subsets formed. In case the balance between T helper and T suppressor is altered then chronicity can be induced.

Cytokines which are responsible for proliferation of the various involved cells have been demonstrated in the inflammatory exudates.

4. In chronic inflammation there is an increase in the number of mononuclear cells such as macrophages and lymphocytes at the site of inflammation. There is also stimulation of antibody formation and immune complexes. As a result, they attract additional cells in order to facilitate and amplify the ongoing chronic inflammatory response.

GENERAL FEATURES OF CHRONIC INFLAMMATION

Through there may be difference in chronic inflammation response depending upon the tissue involved and causative organisms, there are some basic similarities amongst various types of chronic inflammation. Following general features characterize any chronic inflammation

1. MONONUCLEAR CELL INFILTRATION: Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated giant cells. The macrophages comprise the most important cells in chronic inflammation. These may appear at the site of chronic inflammation from

i) Chemotactic factors and adhesion molecules for continued infiltration of macrophages

- ii) Local proliferation of macrophages; and
- iii) Longer survival of macrophages at the site of inflammation.

The blood monocyte on reaching the extravascular space transform into tissue macrophage. Beside the role of macrophage in phagocytosis, they may get activated in response to stimuli such as cytokines (lymphokines) and bacterial endotoxins. On activation, macrophages release several biologically active substances e.g. acid and neutral proteases, oxygen-derived reactive metabolites and cytokines. These products bring about tissue destruction, neovascularisation and fibrosis.

Other chronic inflammatory cells include lymphocyte, plasma cells, eosinophils and mast cells. In chronic inflammation, lymphocytes and macrophages influence each other and release mediators of inflammation.

2. TISSUE DESTRUCTION OR NECROSIS: Tissue destruction and necrosis are central feature of most forms of chronic inflammatory lesions. This is brought about by activated macrophages which release a variety of biological active substance e.g. protease, elastase, collagenase, lipase, reactive oxygen radicals, cytokines, angiogenesis growth factor etc.

3. PROLIFERATIVE CHANGES: As a results of necrosis, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of inflammatory granulation tissue, eventually, healing by fibrosis and collagen laying takes place.

SYSTEMIC EFFECT OF CHRONIC INFLAMMATION

Chronic inflammation is associated with following systemic features

- 1.Fever.** Invariably there is mild fever, often with loss of weight and weakness.
- 2.Anaemia.** Chronic inflammation is accompanied by anaemia of varying degree.
- 3.Leucocytosis.** As in acute inflammation, chronic inflammation also has leucocytosis but generally there is relative lymphocytosis in these cases.
- 4.ESR.** ESR is elevated in all cases of chronic inflammation.
- 5.Amyloidosis.** Long-term cases of chronic suppurative inflammation may develop secondary systemic amyloidosis.

TYPES OF CHRONIC INFLAMMATION

Conventionally, chronic inflammation is subdivided into 2 types

1.Non-specific, When the irritant substance produces a non-specific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

2.Specific, When the injurious agent causes a characteristic histologic tissue response e. g. tuberculosis, leprosy, syphilis.

MECHANISM OF INFLAMMATION^[9]

Pharmacological Mechanism

A series of mediator are released sequentially in inflammatory condition. Pain and extravasations of prostaglandin and kinins. Some prostaglandins are pro inflammatory, particularly of E series, while other is anti-inflammatory. This lends support to the homeostatic mechanism.

Biochemical mechanism

Arachidonic acid is present in the esterified form in the 2nd position of glycerol of phospholipids. It is released by the action of phospholipase A2 (PLA2) or by the combined action of phospholipase C (PLC) and diacylglycerol lipase enzymes. The prostaglandins are synthesized from arachidonic acid by the enzyme cyclo-oxygenase.

When PGG2 is converted to PGH2 by prostaglandin hydroperoxidase it leads to the formation of the highly reactive hydroxyl radical, which accounts for the cellular damage during inflammatory lesions. The scavengers of radicals have anti-inflammatory activity.

Cellular Mechanism

PMNs, monocytes, lymphocytes and platelets play major in inflammatory process. First, for a cell to arrive at the site of inflammation, it has to move towards the vessel wall (margination), adhere to the vascular endothelium site of inflammation (chemotaxis).

The initial vasodilation in case of acute inflammation causes slowing of blood flow and thus helps in margination. The activation of polymorphonucleocytes and monocytes creates a sticky vessel wall surface, which may be due to thromboxane A2. Since the macrophages are rich in tissue thromboplastin the coagulation system also facilitates adherence. The adhesion of PMN to the vascular endothelial cells requires the interaction between adhesion molecules on the endothelial cells. (e.g. Selection and ICAM (intercellular adhesion molecules families.) with corresponding molecules on the neutrophil (e.g. the integrin family).

Inflammatory exudates are also responsible for adherence. The complement component G5a and LTB₄ are powerful chemoattractants. The synthetic peptides formyl methionyl leucyl phenyl (FHLPP) is also chemotactic.

PMNs are the first cells to arrive at the site of inflammation and faster whereas the mononuclear phagocytes remain there for longer time. PMNs are responsible for phagocytosing opsonized bacterial particles. The mononuclear phagocytes help in removing the debris following the death of PMNs. Hydrolytic enzymes that are released during phagocytosis are also capable of causing host tissue damage. By reducing the number of PMNs which reach the site of inflammation, acute inflammation can be attenuated but, since the primary purpose of PMNs is phagocytosis of foreign materials, reduction in PMNs migration leads to chronic inflammatory response.

Macrophages play an important role in chronic inflammatory process. Their importance lies in the production of enormous variety of cytokines, enzymes and other bioactive molecules and their capacity for communication with many cells and tissue including fibroblast and vascular endothelium. In addition to phagocytic function, monocytes present the antigen to the immune system, particular to T-lymphocytes. Interleukin-1 (IL-1) produced by the macrophages activates T-lymphocytes which then produce interleukins-2 (IL-2). IL-2. Facilitated differentiation of T Cells into various functional subsets. When the specific T-cells receives the antigenic message then it activates B cell population to form plasma cell which are capable of synthesizing antibody directed towards the antigen.

Neutrophils predominate synovial fluid due to the neutrophil chemotactic factors such as complement fragments, leukotrienes or IL-8 which are present in the fluid. These cells contains destructive enzyme like elastase and collagenase and these are capable of releasing reactive oxygen species. All of them contribute to the inflammatory response and local tissue destruction.

A number of synovial fibroblasts are found in case of rheumatoid arthritis. This is mainly due to the product fibroblast growth factor such as platelets derived growth factor (PDGF), tumors necrosis factor (TNF α), IL-1 and transformation growth factors (TGF β). Fibroblast is the major source of several important products in case of chronic inflammatory response I rheumatoid arthritis. These are cytokines like IL-6, TGF β , colony stimulating factors (CSFs)

and IL-1. The swelling of the surrounding tissues is due to the production of enzymes, prostanoids, collagen and glucosaminoglycans.

PAIN

It has been proposed that pain to be divided into two entities. “Physiological” and “pathophysiological (clinical)”. “physiological pain” describes the situation, in which a noxious stimulus activates peripheral nociceptors, which then transmit sensory information through several relays until it reaches the brain and is recognized as a potentially harmful stimulus. The pathophysiologic process that occur after tissue injury results in a stimulus-response pattern that is quite different from that seen after physiological pain and therefore has been termed “pathophysiologic pain”.

The perception of pain is a complex interaction that involves sensory, emotional, and behavioral factors. The international Association for the Study of pain has defined pain as: “pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” Nociception, neuropathy, and psychological or environmental factors may single, or in combination, contribute to the experience of pain and the person in pain always must be seen in the context of these interacting factors.

The primary afferent nociceptor is the initial structure involved in nociceptive processes. Nociceptors respond to chemical, mechanical, and thermal stimuli and depending on the response characteristics of the nociceptor, stimulation results in propagation of impulses along the afferent fiber towards the spinal cord. Two main fiber types, the faster conducting myelinated A fiber and the slower- conducting unmyelinated C fibers are involved in the transmission of nociception.

Recent studies have shown that damage to a peripheral nerve results in a number of physiologic, morphologic, and biochemical changes that act as a focus of pain in them. It also has been demonstrated that reduction in blood supply to myelinated fibers results in demyelination, which results in the production of ectopic impulses that may be perceived as the sharp, shooting or burning pain in condition such as diabetic neuropathy.

In chronic subacute inflammatory conditions there is continuous release of inflammatory chemomediators such as potassium, serotonin, bradykinin, substance P, histamine along with

products of arachidonic acid metabolism. These chemicals then act to sensitize high-threshold nociceptors and after sensitization, low-intensity stimuli that normally would not cause pain are perceived as painful. This series of events that occur after tissue injury is termed peripheral sensitization and it is characterized by an increased responsiveness to thermal stimuli at the site of injury. It has been found that there is a class of unmyelinated primary afferent fibers that normally do not respond to excessive mechanical or thermal stimuli. In the presence of inflammation and chemical sensitization, however, they become responsive and discharge vigorously, even during ordinary movement. The properties of these receptors still require characterization, but they have been identified in a number of different tissues and species and are termed “silent” nociceptors. Arthritis pain that occurs with flexion and extension may be a result of activation of these normally “silent” nociceptors.^[14]

HERBLE MEDICINE

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of disease and other different unwanted condition in humans. Ayurvedic medicines are largely based upon herbal and herbomineral preparation and have specific diagnostic and therapeutic principle. Inflammation is a disorder involving localization increase in the number of leucocytes and a variety of complex mediator molecules. Prostaglandin are ubiquitous substance that indicate and modulate cell and tissue response involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular disease. Cancer, colonic adenomas and Alzheimer's diseases.^[5]

Medicinal plants are believed to be an important source of new chemical substance with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers. Anti-inflammatory agents, should therefore, be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs.

Herbal medicine as the major remedy in traditional system of medicine, have been used in medical practice since antiquity. The practice continues today because of its biochemical benefits as well as place in cultural beliefs in many parts of world and has made a great contribution toward maintaining human health. A majority of population in developing countries still relies on herbal medicine. The use of herbal medicine has particularly rich tradition among the people of India, China, Egypt and Brazil. Traditional system of medicine

in India like ayurvedic, siddha and unani are also based on the use of herbal medicine. The use of herbal medicine has been increasing in the developing countries in recent years.^[6]

World health organization (WHO) has been defined a traditional system of medicine as the sum of total of all the knowledge and practices, whether explicable or not used in diagnostic, preventions and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down by generation to generation whether verbally or in writing. In another way traditional system of medicine might also be considered as a solid amalgamation of dynamic medical know-how and experience.

WHO estimate that 65-80% of the world population uses traditional medicine as there primary form of healthcare and about 85% of the traditional medicine involve the use of herbal preparation fully aware of the herbal medicine as a valuable answer readily available resources for primary health care and has endorsed there safe and effective use. WHO has evolves guideline for the identification, cultivation, preparation, evaluation, utilization and conversation of herbal medicine and a comprehensive program for the same has been developed.

Advantages of herbal medicine

1. Herbal medicine have along history of use and better patient tolerance as well as public acceptance.
2. Medicinal plants have a renewable source, which is our only hope for sustainable supplies of cheaper medicine for the worlds growing population.
3. Available of medicinal plant is not a problem specially in developing countries like India having rich agro climatic, culture and ethnic biodiversity.
4. The cultivation and processing of medicinal herbal product are environment friendly.
5. Prolong and apparently uneventful use of herbal medicine may offer testimony of their safety and efficacy.
6. Through the world, herbal medicine has provided many of the most potent medicines to the cast arsenal of the drug available to modern medicinal science, both in crude form and as chemical modern medicine are manufactured.

Limitation if herbal system: Like any other branch of science and technology, presents scenario of herbal medicine has its own limitation arising out its own technical constrains. The prominent limitations of herbal medicines can be summarized as

1. Ineffective in acute medicinal care

As may be observed, herbal medicines are not very effective to treat any acute illness. As most of the medicines are designed to work at molecular level of physiology, the drug takes its time to deliver the result. However there is few herbal medicine which works instantly in acute condition like diarrhea. On the other hand, modern system of medicine has adequate paraphernalia for management of acute conditions. It has already been established by virtue of its efficacy. It may be futile exercise to investigate and discovers such method of acute medical care within the framework of herbal medicine.

2. Inadequate standardization and lack of quality specification

This is the most often criticized aspects of herbal medicine. One important fact is that herbal preparation is administered for its holistic value. Each herbal ingredient in the herbal preparation has an array of chemical constituent with complex molecular formulae. Thus each herbal preparation is a source of polypharmacy within itself. As a result standardization of herbal preparation or its ingredients become a highly complex issue. Standardization of herbal drug by known marker compound may be a complete answer. Despite this major limitation, pharmaceutical industry strives hard to 'in-house' specification based on the quantification of the marker compound. Therefore a consensus is being arrived at, to incorporate the qualitative fingerprinting together with other physiochemical parameter of the herbal drug identification. Development of quality protocols for herbal medicine is an ongoing process and this shortcoming could be overcome shortly.

3. Lack of scientific data and need of re-evaluation

Literature on herbal medicine lack scientific data in support of the medicinal activity claimed and there safety assumed. Hence there is a need to incorporate certain parameters of the pharmacological evaluation of herbal medicine on modern lines.

WHO guidelines clearly direct that it's not necessary to carry out the detailed toxicological evaluation of herbs and herbal preparation originating from traditional system of medicine. Length of continuity on there use is to be taken as testimony of there safety of herbal medicine, few carefully devised clinical studies should be convincing.

3. Stability problem of herbal preparation and need for their formulation development

Most of the herbal preparation are often presented in forms, which are not acceptable as pharmaceuticals formulations as regards to there stability, organoleptic properties,

formulation properties, aesthetic appeal and patient acceptability. Hence it is mandatory to formulate the herbal preparation into suitable dosage form by applying principles of formulation and modern techniques of manufacture.

Despite these limitations, there has been a great fascination for the use of herbal medicine all over the world in past few decades. To keep this ever increasing trend alive, it is imperative to obtain and maintain the quality and purity of herbal raw material as well as finished herbal products on internationally recognized guidelines. The need of the hour is to subject them to rigorous modern scientific testing and processing in order to regulate their quality and purity.

Hence it immediately becomes obvious to apply framework of principles to herbal raw material and finished herbal products like;

- a. Development of reproducible standardisation and quality control technique for herbal ingredients as well as finished herbal products.
- b. Optimization and development of suitable formulation of the given herbal drug/preparation and evaluation of their quality, purity, efficacy, safety and stability.
- c. Enforcement of suitably adapted GMP to ensure the quality of herbal products.
- d. Validation of herbal raw material, finished herbal products and processes.

PLANT DRUG PROFILE^[15, 16, 17, 18]



Biologic name : *Justicia gendarussa*

Family : *Acanthaceae*

Synonyms

Hindi : Nili nargandi

Kannada : aduthodagidda, karalakkigidde

Bengal	: jagatmadan
Tamil	: karunochi, vadaikkutti
Telugu	: addasaramu, gandharasamu
Marathi	: tev, bakas
Sanskrit	: bhutakeshi, gandharasa

Habitat

It is found throughout India, This plant is well distributed in Pakistan, India, Sri Lanka, Indo-China and China.

Chemical constituent

It contain simple aromatic amine, ursolic acid and 2- aimno benzyl alcohol

Uses

The leaves and tender shoots are diaphoretic and used in chronic rumatisum. Fresh leaves are used to treat edema and earache. The plant has been used by the native medical practitioners and tribes to treat various ailments including liver disorders, tumours, inflammation and skin disease. *Justicia gendarussa* has in vitro HIV type 1 reverse transcriptase inhibitory activity.

LITERATURE REVIEW

1. Ratnasooriya, W. D., studied Antinociceptive activity and toxicological study of aqueous leaf extract of *justicia gendarussa* Brum. F. in rats.
2. Mruthunjaya, K., studied Antioxidant and free radical scavenging potential of *Justicia gendarussa* leaves in vitro.
3. Gangadevi, K., caeeied Isolation of colletotrichum gloeosporioides, a novel endophytic toxal- producing fungus from the leaves of medicinal plants, *Justicia gendarussa*.

OBJECTIVES

Inflammation induce pain have affected mankind for ages. Their crippling and incapacitating effect on the affected patient present many emotional, social and economical problems. Despite lot of research and effective cure has still eluded us. All that can be offered are the anti-inflammatory and pain killer like NASID. Ayurvedic literature is full of herb for solving this type of pain.

The specific objective of this project is,

- Selection of suitable herbs and to develop a suitable solvent system for giving herbal dry powder extract.
- To evaluate in vivo anti-inflammatory and analgesic activity of the extracts.

PLAN OF WORK

- ▶ Collection, Drying and grinding of leaves.
- ▶ Authentication of the leaves.
- ▶ Determination of physiochemical parameter.
- ▶ Extraction of leaves with different solvents.
- ▶ Procurement of animals and their diet.
- ▶ Evaluation of extracts in animals for their,
 - i) Anti- inflammation and activity
 - ii) Analgesic activity
- ▶ Procurement of chemical reagent for estimation of Anti- inflammation and Analgesic activity.
- ▶ Interpretation of result by statistical analysis.

1. MATERIALS

1.1. Plant material collection and authentication

The *Justicia gendarussa* leaves were collected from Nagpur District, Maharashtra, India In the month of September 2009. The plant was identified and authenticated by R M Acharya, Post graduate teaching Department of Botany, Wardha, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur. A voucher specimen No.9409 was deposited with the post graduate Teaching Department of Botany, Wardha, Nagpur University, Nagpur.

1.2. Procurement of Experiment animals

The animals bred in the animal house of Institute of Pharmaceutical Education and Research, Wardha were procured for the experiment. The animal were house in polypropylene cages at a temperature of $25 \pm 2^{\circ}\text{C}$ with relative humidity of 40-60% and 12:12 hour light dark cycle. Animal were fed with a balance diet and water *ad libitum* during the complete experiment period. All animal experiment were approved by the Institutional Animal Ethical Committee (Registration No. 535/02/a/CPCSEA/Jan2002) of Institute of Pharmaceutical Education and Research, Wardha.

1.3 Instrument

Hot plate method (Eddy and Leimbach, 1953)

Pleythysmometer (Almemo, 2290-4)

2. METHODS

2.1. Drying and size reduction of *Justicia gendarussa* leaves

The leaves of *justicia gendarussa* were dried in shed and powered to $\neq 22$ mesh size, stored in the airtight container till further use.

Extraction of *Justicia Gendarussa* leaves

The shade dried and powered leaves of *justicia gendarussa* leaves, were subjected to successive extraction in a soxhlet apparatus with petroleum ether (60-80°), chloroform, methanol and finally macerated with water so as to get respective extract. All extracts were individual filtered, through Whatsman filter paper ± 42 and evaporated to dryness at 50°C in oven. The extracts were then stored in desiccators till further use.⁷²

2.2.1. Extraction process

Soxhlet apparatus was used for continuous extraction of the powdered crud drug. The material was packed in the apparatus and allowed to get extracted with hot solvent that continuously percolates from top to bottom. Condensed fresh solvent percolates every time through the powder and is the major advantage with this technique. The powder was extracted using solvents petroleum ether (60-80°), chloroform and methanol respectively for 24 hrs. The ratio of powder to solvent was 10:100.

2.2.2. Petroleum ether (60-80°) extraction

Dried powder was charged in soxhlet apparatus and first extracted with petroleum ether to remove fatty material. After the extraction process, solvent was distilled off and the extract was dried at 50°C. Dried extract was stored in desiccators till further use.

2.2.3. Chloroform extraction

Mark obtained from petroleum ether extraction was air dried and extracted with Chloroform. After the extraction process, solvent was distilled off and the extract was dried at 50°C. Dried extract was stored in desiccators till further use.

2.2.4. Methanol extraction

Mark obtained from Chloroform extraction was air dried and extracted with Methanol. After the extraction process, solvent was distilled off and the extract was dried at 50°C. Dried extract was stored in desiccators till further use.

2.2.5. Water extraction

Mark obtained from Methanol extraction was air dried and extracted with macerated at room temperature. The extract was dried at 50°C. Dried extract was stored in desiccators till further use.

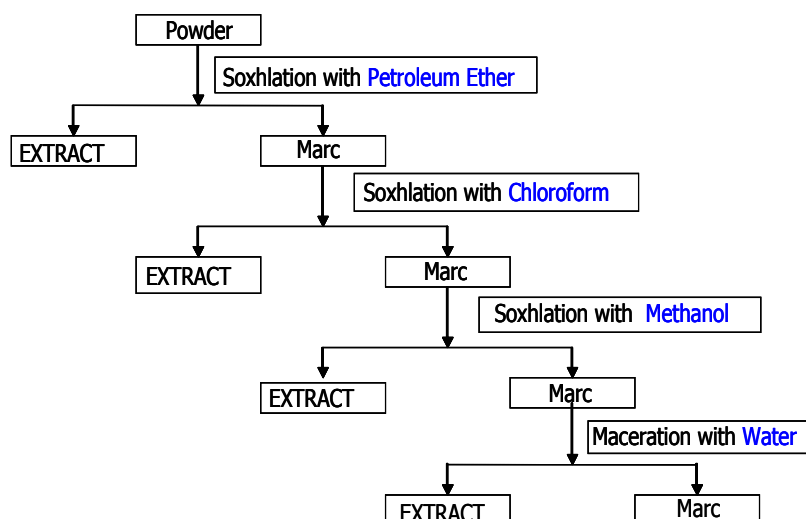


Fig. No. 1: Flow chart showing extraction process of *Justicia gendarussa* leaves

Table No.2: Extractive values of *Justicia gendarussa* leaves extracts.

Sr. NO	Solvent	Extraction Process	% Yield
1	Petroleum ether(60-80°) (PEE)	Soxhlation	8.2%
2	Chloroform (CLE)	Soxhlation	6.5%
3	Methanol (MEE)	Soxhlation	12.3%
4	Water (WAT)	Maceration	18%

2.4. Acute oral toxicity test in rat: fixed dose procedure according to OECD 420 guidelines

2.4.1. Aim and objective of the test

The aim of the test is to obtain using a minimum no of animals, sufficient information on the acute toxicity after single administration, by oral rout in the rat, of a test substance, for its classification.

Test substance administered to a group of experiment animals by oral route at one defined dose (5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg) according to the available information on test substance. The liquid preparation was given less than 1 ml/100 gm body wt. of animals. Animals were observed after one hour at least, after administration to detect signs of toxicity.

2.4.2. Animals used

Male albino rats, weighing 150-200 gm were used. They were housed in the standard environmental condition and fed with diet with water. The animals were housed in 37cm × 23cm × 16cm polypropylene cages with maximum of 6 animals per cage. The cages were placed in limited access premises of animal house with controlled temperature and humidity. The artificial lighting ensured a sequence of 12 hours light and 12 hours dark.

2.4.3. Test procedure

Carrageenan- induced rat paw edema

Animals were fasted for 24 h before the experiment with free access to water. Approximately 0.1 ml of a 1% suspension of carrageenan in saline was prepared 1 h before each experiment and was injected into the planter side of right hind paw of rat. The rats were divided into six groups (n=6). Group 1 served as control and received normal saline and the groups 2, 3 and 4 were treated orally with aq extract, pet ether and ethanol extract 250-500 mg/kg b.w., respectively. Group 5 received the standard drug aceclofenac (10 mg kg⁻¹) the aq extract, pet ether and ethanol extract was administered 1 h prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub planter of each rat. The paw volume was measured initially and then at 1, 2, and 3 h after the carrageenan injection by using plethysmometer. The anti-inflammatory effect was calculated by the following equation

$$\text{Anti-inflammatory activity (\%)} = (1 - V_t/V_c) \times 100$$

Where, V_t represents the paw volume in drug treated animals and V_c represent the paw volume of control group animals.

Analgesic activity

Hot plate method: The analgesic activity of different extract was assessed using as described by hot plate method of Eddy and Leimbach (1953). The evaluated parameters were the

latency time for paw licking and jumping responses on exposure to the hot plate surface which is kept at $55\pm 1^{\circ}\text{C}$. The animals were kept in the hotplate until it lifted one of its hind paws. For this method, the rats were divided into 6 groups of 6 animals each. Group I served as control (5% gum acacia, 1 mL 100 g^{-1}), group II, III and IV received aqueous extract, petroleum ether extract and ethanol extract at a dose of 300 and 1500 mg kg^{-1} orally. Group V received Aspirin at a dose of 25 mg kg^{-1} . All the treatments were given 30 min before the thermal stimulus and the response was determined at 60, 120 and 180 min.

Table No. 1: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '0' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg^{-1}	0.252 ± 0.020
Aceclofenac	10 mg kg^{-1}	0.220 ± 0.021
WTE	1500 mg kg^{-1}	0.246 ± 0.024
PEE	300 mg kg^{-1}	0.243 ± 0.022
ETE	300 mg kg^{-1}	0.236 ± 0.018

N=6. P Value is not significant. Data were analyzed by one way ANOVA followed by Dunnett test

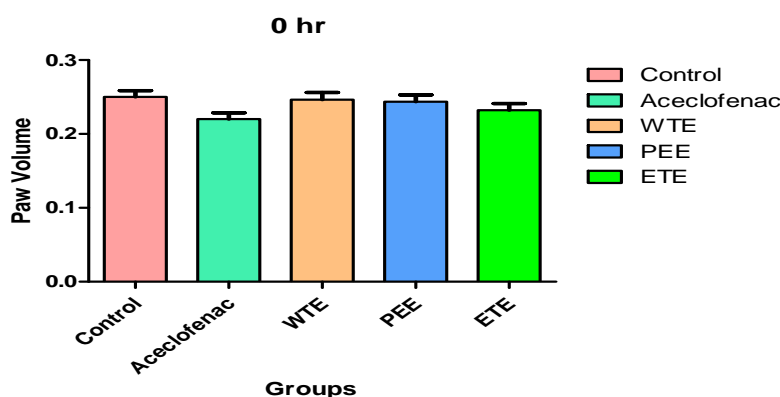


Fig.no.1. Graph showing change in the paw volume in '0'hr

Table No. 2: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '1' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg^{-1}	0.273 ± 0.027
Aceclofenac	10 mg kg^{-1}	0.238 ± 0.016
WTE	1500 mg kg^{-1}	0.247 ± 0.023
PEE	300 mg kg^{-1}	0.250 ± 0.021
ETE	300 mg kg^{-1}	0.230 ± 0.027

N=6. P Value is not significant. Data were analyzed by one way ANOVA followed by Dunnett test

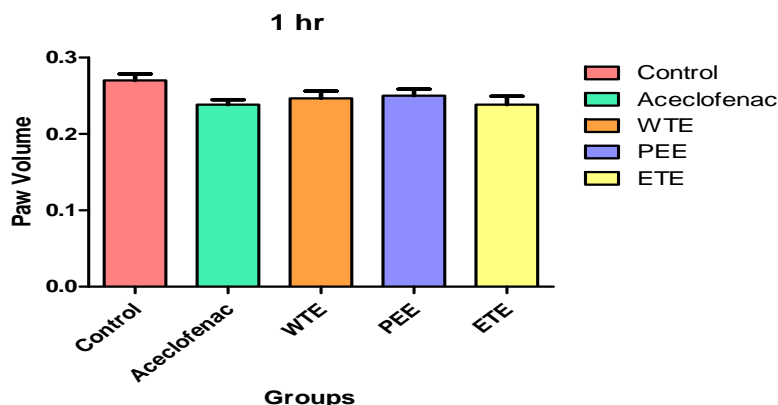


Fig no. 2 Graph showing change in the paw volume in '1' hr

Table No. 3: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '2' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg ⁻¹	0.440±0.021
Aceclofenac	10 mg kg ⁻¹	0.260±0.022*
WTE	1500 mg kg ⁻¹	0.275±0.024*
PEE	300 mg kg ⁻¹	0.240±0.022*
ETE	300 mg kg ⁻¹	0.250±0.023*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test.

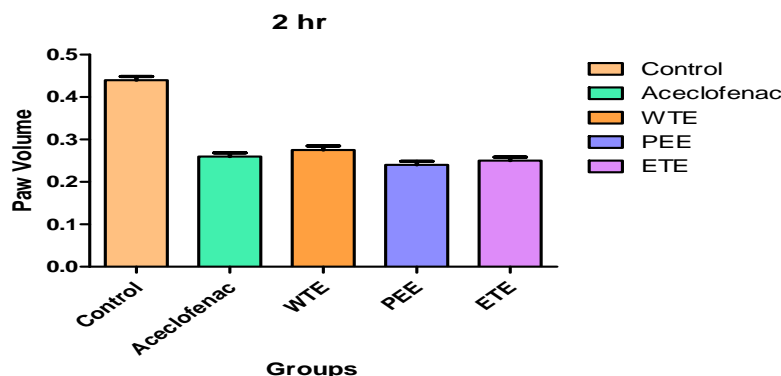


Fig no.3 Graph showing change in the paw volume in '2' hr

Table No. 4: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '3' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg ⁻¹	0.460±0.021
Aceclofenac	10 mg kg ⁻¹	0.260±0.022*(47%)
WTE	1500 mg kg ⁻¹	0.280±0.043*(39%)
PEE	300 mg kg ⁻¹	0.290±0.024*(37%)
ETE	300 mg kg ⁻¹	0.258±0.021*(44%)

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test.

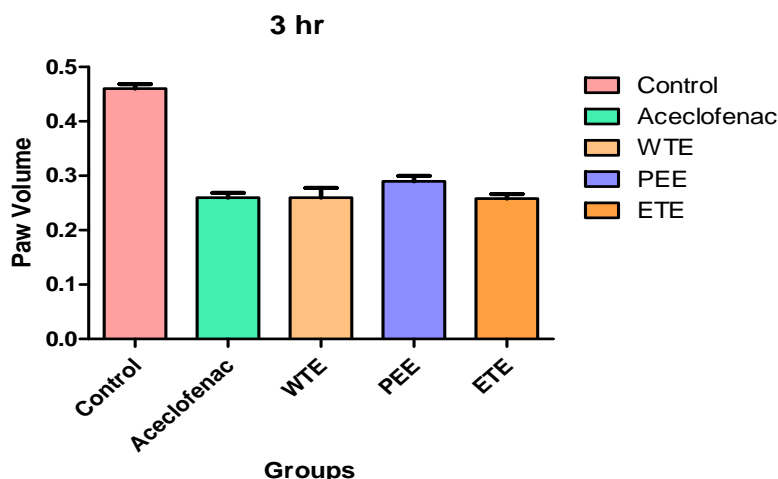


Fig no. 4 Graph showing change in the paw volume in '3' hr

Table No. 5: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '0' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg ⁻¹	2.320±0.037
Asprin	25 mg kg ⁻¹	2.570±0.183
WTE	1500 mg kg ⁻¹	2.370±0.0284
PEE	300 mg kg ⁻¹	2.420±0.0037
ETE	300 mg kg ⁻¹	2.480±0.047

N=6. P Value is not significant. Data were analyzed by one way ANOVA followed by Dunnett test

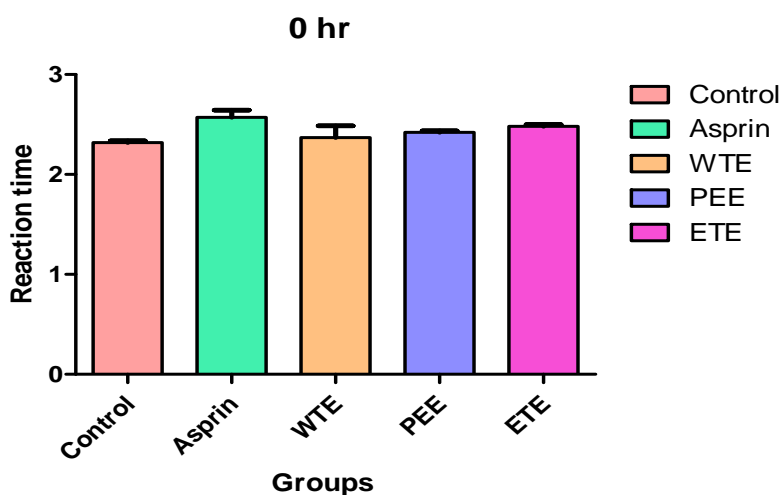


Fig no. 5 Graph showing change in reaction time in '0' hr

Table No. 6: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '1' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg ⁻¹	2.252±0.078
Asprin	25 mg kg ⁻¹	9.058±0.601*
WTE	1500 mg kg ⁻¹	3.677±0.647s*
PEE	300 mg kg ⁻¹	3.818±0.029*
ETE	300 mg kg ⁻¹	6.925±0.729*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

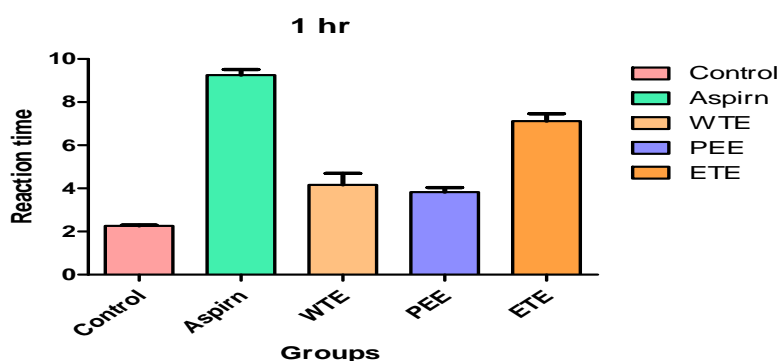


Fig no. 6 Graph showing change in reaction time in '1' hr

Table No. 7: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '2' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg ⁻¹	2.445±0.360
Asprin	25 mg kg ⁻¹	9.912±0.900*
WTE	1500 mg kg ⁻¹	4.677±1.992*
PTE	300 mg kg ⁻¹	5.158±1.634*
ETE	300 mg kg ⁻¹	7.835±0.728*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

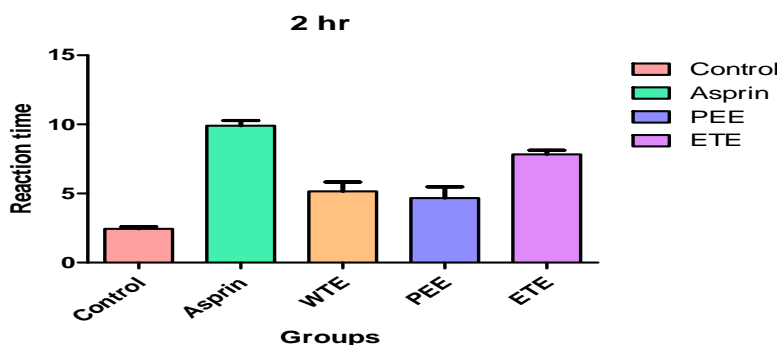


Fig no. 7 Graph showing change in reaction time in '2' hr

Table No.8: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '3' hr

Treatment	Dose	Treatment
Control	10 mL kg ⁻¹	2.312±0.023
Asprin	25 mg kg ⁻¹	8.790±0.021*
WTE	1500 mg kg ⁻¹	4.517±0.240*
PEE	300 mg kg ⁻¹	4.853±0.132*
ETE	300 mg kg ⁻¹	6.487±0.252*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

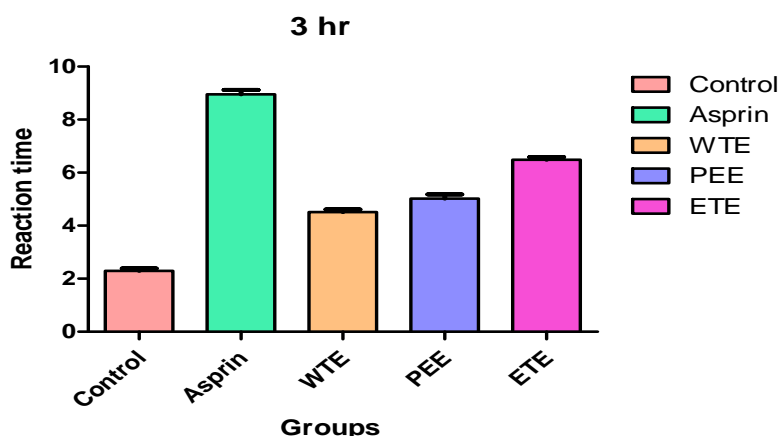


Fig no. 8 Graph showing change in reaction time in '3' hr

Phytochemical screening: Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids. Glycosides, triterpenes, flavonoids and phenolic compounds. Further separation of the specific phytochemical is in progress.

Acute toxicity studies (LD₅₀): The extract treated animals were observed for mortality up to 48 h (short term toxicity) and for long- term toxicity (14 days). The study indicated that the different extract did not produce any mortality up to 2000 mg kg⁻¹.

Effect of different extract on carrageenan induced rat paw edema: The result of different extract against carrageenan-induced paw edema is shown in 0,1,2 and 3 hr. In 0 and 1 hr there is no significant p value but in 3 and 4 hr gave significant (p<0.001) reduction of rat paw edema at all assessment times in a dose dependent manner. The extract showed maximum inhibition of 44% at the dose of 300 mg kg⁻¹ after 3h of drug treatment in carrageenan-induced paw edema whereas the standard drug showed 47% of inhibition.

Effect of different extract on hot plate method: The animals pretreated with different extract showed a dose dependent increase in latency of responses in the hot plate method. The increase in the latency responses were significant ($p < 0.001$). When compared to control. At time interval of 3h, 300 and 1500 mg kg⁻¹ different extract of *Justicia gendarussa* effect were found to be decreased remarkably compared with standard drug. In '0' hr there is no significant p value but in 1,3 and 4 hr gave significant ($p < 0.001$) However, their effects are equal during the second hour of experiment.

DISCUSSION

The screening of bioactive agents from plants is one of the most intensive areas of natural products research today; literature showed that only 10% of all plant have been investigated in detail for bioactive agents.

Literature survey of *Justicia gendarussa burm.* releaved the presence of alkaloids. Glycosides, triterpenes, flavonoids and phenolic compounds. Futher separation of the specific phytochemical is in progress.

The result of different extract against carrageenan-induced paw edema is shown in 0,1,2 and 3 hr. In 0 and 1 hr there is no significant p value but in 3 and 4 hr gave significant ($p < 0.001$) reduction of rat paw edema at all assessment times in a dose dependent manner. The extract showed maximum inhibition of 44% at the dose of 300 mg kg⁻¹ after 3h of drug treatment in carrageenan-induced paw edema whereas the standard drug showed 47% of inhibition.

The animals pretreated with different extract showed a dose dependent increase in latency of responses in the hot plate method. The increase in the latency responses were significant ($p < 0.001$). When compared to control. At time interval of 3h, 300 and 1500 mg kg⁻¹ different extract of *Justicia gendarussa* effect were found to be decreased remarkably compared with standard drug. However, their effects are equal during the second hour of experiment.

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced rat paw edema. The development of edema in the paw of the rat after injection of carrageenan is believed to the biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin; the second is due to the release of prostaglandin-like substance. Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and

serotonin and the action in the second phase may be explained by an inhibition of cyclooxygenase.

Ueno et al. (2000) found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates. Besides, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism. The extract produced minimum inhibition in the initial phase of development of inflammation. The standard drug, aceclofenac, showed inhibition in the second phase. Therefore, it is suggested that the mechanism of action of extract may be related to prostaglandin synthesis inhibition.

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, whereas, local anesthetics and narcotics do. However, the hot plate test was undertaken to verify if extract would have any certain analgesic effect. The results for the group treated with extract showed significant activity when compared to control group and nearly equal to the group treated with aspirin (25 mg/kg). Hence, it is assumed that extract has significant central analgesic effect.

CONCLUSION

Data obtained in this study indicated that the extract of leaves of *Justicia gendarussa* possesses anti-inflammatory and analgesic effects. The presence of flavonoids might be responsible for these activities and which are probably mediated via inhibition of various autacoids formation and release. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

Future scope

The present work may be extended for

- Further evaluation of anti-inflammatory activity of *Justicia gendarussa* leaves extracts using various methods.
- Further fractionation of active extract of *Justicia gendarussa* leaves for isolation of active principle constituent.
- Identification and characterization of active plant metabolites responsible for anti-inflammatory activity.
- Evaluation of anti-inflammation activity by taking extracts in other solvents.

ACKNOWLEDGEMENT

It is a moment of happiness for me and my family as the dream seen has come true with completion of the degree of Master of Pharmacy. I thank the almighty God who all the time blessed me with the good friends and guided me the right path to achieve this destination. I feel immense pleasure to acknowledge my profound sincere sense of gratitude for valuable guidance, keen interest and encouragement right from selection of dissertation topic up to final shaping of the thesis towards my guide **Mr. C.R Tenpe**, Assistant Professor, IPER Wardha. It is my privilege to work under his able and inspiring guidance. I therefore feel indebted to him.

It is my privilege to acknowledge my deep indebtedness to **Prof. (Dr.) P. G. Yeole**, Principle I.P.E.R. Wardha for providing the good facility and the requirements to complete the dissertation work.

I am deeply indebted to Botany department R.T.M. Nagpur University for their proper authentication of plant for my project work. I express my sincere thanks to Mr. Dharpure and Mr. Gunvanta for their co-operation during the tenure of the research work.

I am also thankful to my friend and colleagues for supporting me, directly or indirectly during completion of my project work. I am also thankful to Mr. Wasudeo K. Tajane for helping me in compilation, editing and printing of this thesis.

Last but not least, I wish to express my heart thanks to my parents, brother, husband, my son and friends who always encouraged me at every moment of my life. I shall remain to them forever.

May the almighty continue to support me till the last destination of endless education.

Place: Wardha,

Date:

Miss. Jayshree R. Aate

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