ANTI-INFLAMMATORY EFFECT OF AQUEOUS EXTRACT OF LEAVES OF *Oryza sativa* Linn IN *IN-VITRO* ENZYME ASSAYS AND CARAGEENAN INDUCED PAW EDEMA IN WISTAR RATS

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai-32

In partial fulfillment of the award of the degree of

MASTER OF PHARMACY IN
PHARMACOLOGY

Submitted by
REG.No. 261425229

Under the Guidance of
Dr. V. RAJESH, M.Pharm,Ph.D.,

DEPARTMENT OF PHARMACOLOGY
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KUMARAPALAYAM– 638 183
TAMILNADU

OCTOBER-2016

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Anti-inflammatory Effect of Aqueous Extract of Leaves of *Oryza sativa* linn in *In-vitro* Enzyme Assays and carageenan Induced Paw Edema in Wistar Rats"** submitted by the student bearing **[REG.No. 261425229]** to **"The Tamil Nadu Dr. M.G.R.Medical University"**, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on.....

Internal Examiner **External Examiner**

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This is to certify that the dissertation **"Anti-inflammatory Effect of Aqueous Extract of Leaves of *Oryza sativa* linn in *In-vitro* Enzyme Assays and carageenan Induced Paw Edema in Wistar Rats"** is a bonafide work done by **Reg.No. 261425229,** Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, in partial fulfillment of the University rules and regulations for award of **Master of Pharmacy** in **Pharmacology** under my guidance and supervision during the academic year 2015-2016.

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DECLARATION

I do hereby declared that the dissertation**"Anti-inflammatory Effect of Aqueous Extract of Leaves of *Oryza sativa* linn in *In-vitro* Enzyme Assays and carageenan Induced Paw Edema in Wistar Rats"** submitted to "**The Tamil Nadu Dr.M.G.R Medical University"**, Chennai, for the partial fulfillment of the degree of **Master of Pharmacy** in **Pharmacology,** It is a bonafide research work has been carried out by me during the academic year 2015-2016, under the guidance and supervision of **Dr. V. RAJESH, M.Pharm,Ph.D.,** Professor, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma ,associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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महोदया/Madam,

The plant specimen brought by you for identification is identified as *Oryza sativa* L. - GRAMINEAE

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

(डॉ. जी.वी.एस. मूर्ति /Dr.V. Murthy)
वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष /
Scientist 'F' & Head of Office

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Animal Ethical Committee Clearance Certificate

We, the Undersigned Chairman/Members of the Animal Ethical Committee, functioning in JKK Nattraja College of Pharmacy have studied the proposed research Subject/Project of **SHANMUGASUNDARAM E.C** titled **"Anti-inflammatory Effect of Aqueous Extract of Leaves of Oryza sativa linn in In-vitro Enzyme Assays and carageenan Induced Paw Edema in Wistar Rats"** applying for permission for animal usage and hereby give the certificate of clearance of approval by this Ethical Committee.

Signature of the Chairman/ Members of the Animal Ethical Committee

Name of the Institution:

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Date :

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SHANMUGASUNDARAM.E.C

[REG.No. 261425229]

INDEX

1. INTRODUCTION

Inflammatory disease is one of the major health problems worldwide. Inflammation is a fundamental response of virtually all multicellular organisms to infection and injury. Functions of the inflammatory response include detection and response to invading microbes and to cellular damage and repair of tissue injury. Both deficiencies and dysregulation of inflammation underlie many common and life-threatening clinical disorders including asthma or bronchial hyperreactivity, infection, sepsis and atherosclerotic disease, which all have a substantial societal impact.[1]

Oxidative damage of cellular biomolecules such as proteins and lipids is thought to play a crucial role in the incidence of several chronic inflammations.[2] It is among causative factors in induction of many chronic and degenerative diseases including artherosclerosis, ischemic heart disease and arthritis. Arthritis disease generates many free radicals which maintain inflammations as long as possible.

OCCURRENCE

Inflammation is a major condition associated with various diseases. Rheumatoid arthritis is one of the challenging disorders associated with inflammatory condition.[3] Various molecules have been proven very effective in such condition. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone.

All of these drugs present well known side and toxic effects. It is well documented that these non-steroidal anti-inflammatory drugs (NSAIDs) produce intestinal tract ulcers (With potential internal bleeding) in 10-30 % of long-term users, and erosions of the stomach lining and intestinal tract in 30-50 % of cases.[4] As a result of these side effects, NSAID use is associated with 10,000-20,000 deaths per year in the U.S.[5] Even the new COX-2 inhibitor drugs only been reported to reduce intestinal tract damage by 50 %, and their toxicity to the liver and kidney is still under review.[6]

Inflammation is the common underlying thread that runs through many clinical conditions; Research in this area requires a multi-dimensional approach encompassing various fields of medical sciences, basic sciences and clinical informatics. Considering this, a two-day

scientific meet on inflammation was organized in Bengaluru recently. It brought together researchers from clinical and basic sciences working in the field of inflammation from various parts of the country. The purpose of the meet was to initiate a crosstalk on the role of inflammation in non-communicable diseases. While the meeting offered a platform for a multi-disciplinary approach to strengthening inflammation research in India, the deliberations in a nutshell are presented here.

The meet started with a talk by S. Chandrashekara (ChanRe Rheumatology and Immunology Centre and Research (CRICR), Bengaluru) on 'Dichotomy of quantifying and managing the inflammation in autoimmune disease'. He deliberated on the need 'to quantify, qualify and assess the impact' of clinical and inflammatory markers to regulate inflammation in autoimmune diseases.[7] He highlighted the dilemma faced by clinicians in deciding when to stop the inflammation and thereby the damage produced by inflammation, how much to control and how to control the inflammatory process in autoimmune disease management by taking rheumatoid arthritis (RA) as a prototype disease. He further shared the usefulness of different lines of treatment for different forms of uveitis and scleritis patients. He cautioned about the drug-related adverse effects, the need to follow up with investigations every two weeks and to discontinue in case of side effects in patients on therapy.

INFLAMMATION

Inflammation is the response of living tissue to injury. It involves a well-organized cascade of fluid and cellular changes within living tissue.

The four principal effects of inflammation (rubor, tumor, caloret dolor) were described nearly 2,000 years ago by the Roman Aulus Cornelius Celsus, more commonly known as Celsus. (He wasn't actually a practitioner of medicine. Rather, he wrote an encyclopedia that had many volumes about all kinds of subjects. Only the volume concerning medicine survived).

Redness (rubor)

An acutely inflamed tissue appears red, due to dilatation of small blood vessels within the damaged area (hyperemia).

Swelling (tumor)

Swelling results from edema, the accumulation of fluid in the extravascular space as part of the inflammatory fluid exudates and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the area.

Heat (calor)

Increase in temperature is readily detected in the skin. It is due to increased blood flow (hyperemia) through the region, resulting in vascular dilation and the delivery of warm blood to the area.

Pain (dolor)

Pain results partly from the stretching and distortion of tissues due to inflammatory edema and in part from some of the chemical mediators of acute inflammation, especially bradykinin and some of the prostaglandins.

Loss of function (functiolaesa)

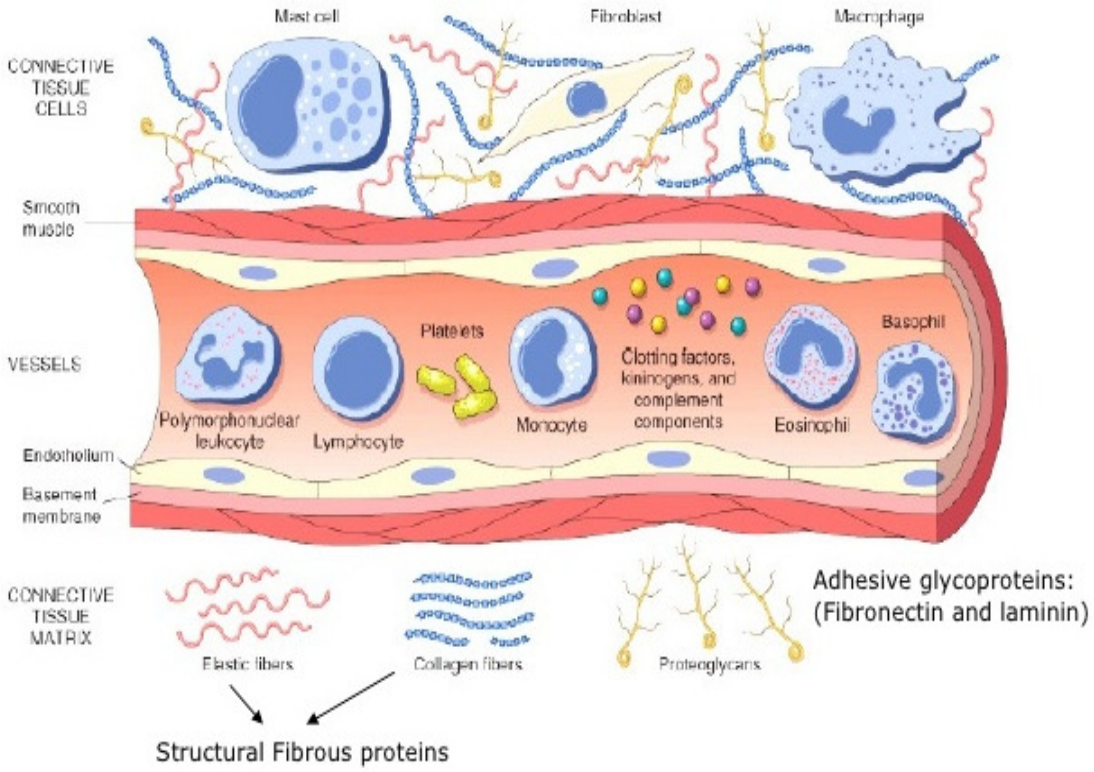
Loss of function, a well-known consequence of inflammation, was added by Virchow (1821-1902) to the list of features described in Celsus' written work. Movement of an inflamed area is inhibited by pain, either consciously or by reflexes, while severe swelling may physically immobilize the affected area.

CAUSES OF INFLAMMATION

Microbial infections

One of the most common causes of inflammation is microbial infection. Microbes include viruses, bacteria, protozoa, fungi and various parasites. Viruses lead to death of individual cells by intracellular multiplication, and either cause the cell to stop functioning and die, or cause explosion of the cell (cytolytic), in which case it also dies. Bacteria release specific toxins – either exotoxins or endotoxins. What's the difference? Exotoxins are produced specifically for export (like anthrax toxins or tetanus toxins) whereas endotoxins are just part of the cell walls of Gram negative bacteria and they do terrible things to the body too but they aren't as specific in their actions as the exotoxins.

The components of acute and chronic inflammatory responses: circulating cells and proteins, cells of blood vessels, and cells and proteins of the extracellular matrix.



Hypersensitivity reactions

A hypersensitivity reaction occurs when an altered state of immunologic responsiveness causes an inappropriate or excessive immune reaction that damages the tissues. The types of reaction will be discussed in more detail later (In the lesson on Immune Mediated Inflammation).

Physical agents, irritant and corrosive chemicals

Tissue damage leading to inflammation may occur through physical trauma, ultraviolet or other ionizing radiation, burns or excessive cooling ('frostbite'). Corrosive chemicals (acids, alkalis, oxidizing agents) provoke inflammation through direct tissue damage. These chemical irritants cause tissue damage that leads directly to inflammation.

Tissue necrosis

Death of tissues from lack of oxygen or nutrients resulting from inadequate blood flow (infarction) is a potent inflammatory stimulus. The edge of a recent infarct often shows an acute inflammatory response.

EFFECTS OF INFLAMMATION

The effects of inflammation can be both local and systemic. The systemic effects of acute inflammation include fever, malaise, and leukocytosis. The local effects are usually clearly beneficial, for example the destruction of invading microorganisms, but at other times they appear to serve no obvious function, or may even is harmful.

Beneficial effects of inflammation	Harmful effects of inflammation
Dilution of toxins	Persistent cytokine release
Entry of antibodies	Destruction of normal tissues
Fibrin formation	Swelling
Delivery of nutrients and oxygen	Inappropriate inflammatory response
Stimulation of immune response	

Systemic Effects of Inflammation

Both acute and chronic inflammation, even if well localized, can have effects on the whole body. The main ones are:

Leukocytosis

Leukocytosis is a common feature of inflammatory reactions. Leukocytosis means that there is an abnormally high number of circulating white blood cells. A general rule is thatincreased neutrophils indicate a bacterial infection whereas increased lymphocytes are most likely to occur in viral infections. This is one reason why we often do a CBC when an animal is sick – gives us more clues.

Fever

Fever is a common systemic response to inflammation. Fever is most often associated with inflammation that has an infectious cause, although there are some non-infectious febrile diseases. Fever is coordinated by the hypothalamus and involves a wide range of factors.

TYPES OF INFLAMMATION

It is well known that inflammation is a complex physiological response and may be acute and chronic.[8] Acute inflammation is to be activated when encountering harmful stimuli, which is characterized by not only vasodilatation, permeability accentuation and neutrophils infiltration, but also edema formation.[9] The acute inflammatory response will lead to chronic inflammation that is featured by tissue proliferation, granuloma, and repair.[10]

ACUTE INFLAMMATION

In the early stages of inflammation, the affected tissue becomes reddened due to increased blood flow and swollen, due to edema fluid. These changes are the result of vascular response to inflammation. The vascular events of the acute inflammatory response involve three main processes:

1. Changes in vessel caliber and consequently blood flow (hemodynamics)
2. Increased vascular permeability and
3. Formation of the fluid exudates

1. Changes in Vessel Caliber

The microcirculation consists of the network of small capillaries lying between arterioles, which have a thick muscular wall, and thin-walled venules. Capillaries have no smooth muscle in their walls to control their caliber, and are so narrow that red blood cells must pass through them in single file. The smooth muscle of arteriolar walls forms pre-capillary sphincters that regulate blood flow through the capillary bed. Flow through the capillaries is intermittent, and some form preferential channels for flow while others are usually shut down. In other words, there is not blood flowing through all capillaries all the time. They take turns. When inflammation happens, none of them gets to take their scheduled tea break. They are all open. Experimental evidence indicates that blood flow to the injured area may increase up to ten-fold as vessels dilate. What causes this to happen? MEDIATORS - including nitric oxide, histamine and prostaglandins (PGI_2) and LTB_4.

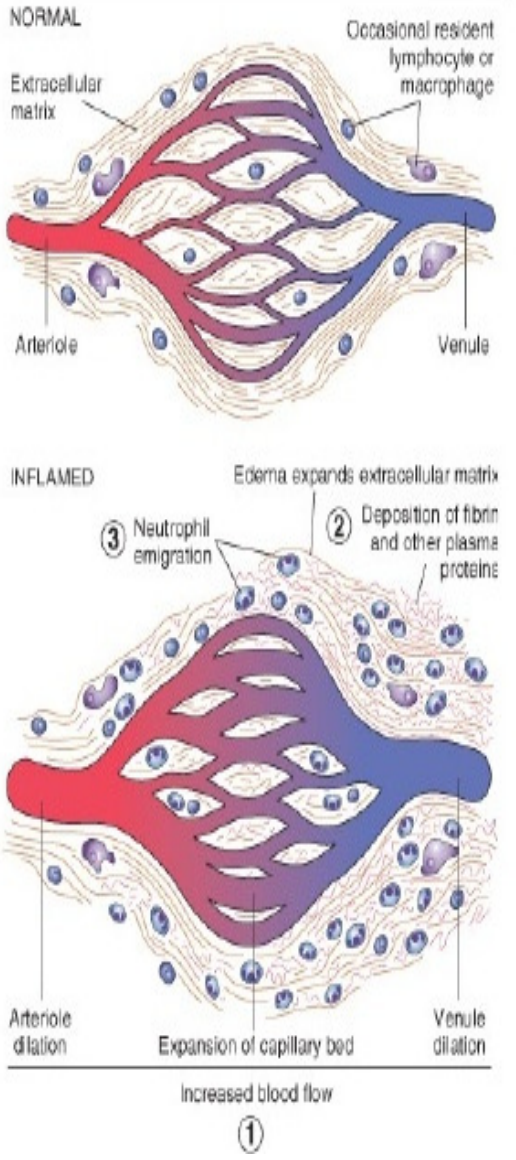
2. Increased vascular permeability

In acute inflammation, the capillary hydrostatic pressure increases, and there is also escape of plasma proteins into the extravascular space due to increased vascular permeability (endothelial contraction allowing proteins to escape between cells). Consequently, much

more fluid leaves the vessels than is returned to them. The net escape of protein-rich fluid is called exudation; hence, the fluid is called an exudate.

Vascular changes play an important role during acute inflammation
(begin early after injury and depends upon the severity of the injury)

- **Vasodilation**, leads to increased blood flow causing redness and warmth (rubor and calor)
- **Increased Permeability**, leads to exudation of protein rich fluid into the extravascular space causing swelling (tumor)
- Loss of fluid from the vessels leads to **Concentration of red cells** resulting in decreased velocity and **stasis** of the blood flow
- **Leukocyte rolling, adhesion and migration** leads to the accumulation of inflammatory cells



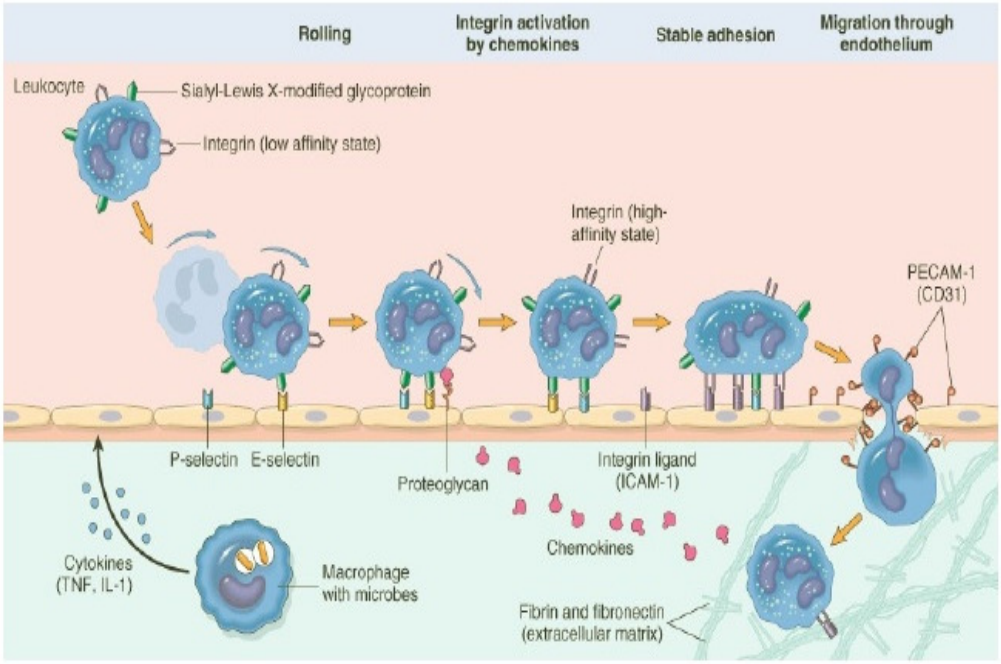
3. Formation of the Cellular Exudate

The movement of leukocytes from the vessel lumen in a directional fashion to the site of tissue damage is called chemotaxis. All granulocytes and monocytes respond to chemotactic

factors and move along a concentration gradient (from an area of lesser concentration of the factor to an area of greater concentration of the factor).

Leukocytes play a very important role in microbial killing. In any inflammatory response, leukocyte activation is a prerequisite to their full participation in the process. Leukocytes become activated during inflammation.

The multistep process of leukocyte migration through blood vessels.



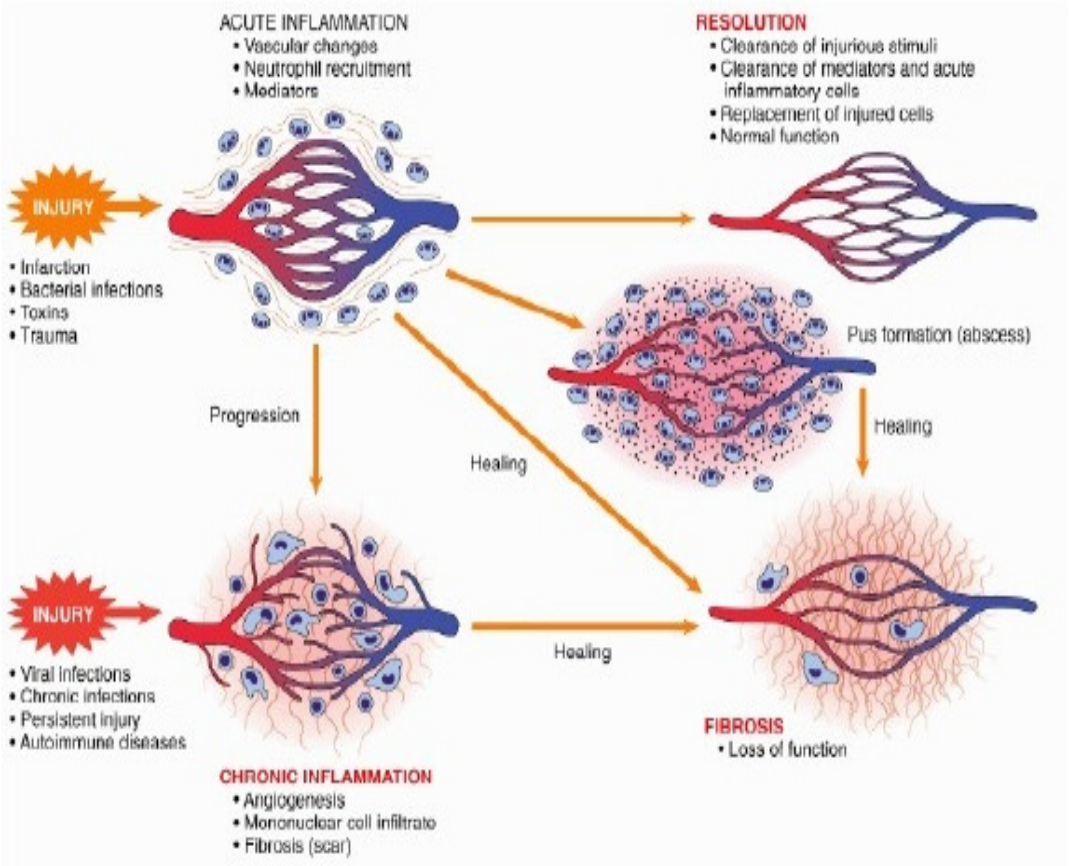
- Slowly move along the endothelium and adhere transiently (process called rolling) finally they come to rest at some point - adhere firmly.
- After adhesion they insert there pseudopods into endothelial cell junction and squeeze through this layer into the extarvascular space.
- The process of adhesion and transmigration is determined by binding of adhesion molecules on leukocytes and endothelial cells.

Leukocyte exudation - Divided into 4 steps:

- Margination, rolling and adhesion to endothelium
- Diapedesis (trans-migration across the endothelium)
- Migration toward a chemotactic stimulus from the source of tissue injury.
- Phagocytosis

CHRONIC INFLAMMATION

Host response to an inciting stimulus that goes on for weeks or months. Chronic inflammation, like its acute cousin, is a host response to an inciting stimulus. There are, however, some distinct differences. First and foremost is the time factor. Chronic inflammation is considered to be inflammation of prolonged duration - weeks to months. Second, rather than being just exudative, chronic inflammation usually is productive or proliferative. Chronic inflammation is rarely gooey. Cells in the chronic inflammatory process tend to produce substances that add new tissue, such as collagen and new blood vessels. Many of these changes also represent the repair process and there is a blurry continuum between chronic inflammation and the whole repair process. In general, chronic inflammation is characterized by inflammation, tissue destruction, and attempts at repair all happening at once.



Grossly, chronic inflammation does not have as much rubor (redness) or calor (heat) as in the acute reaction. Also, exudates aren't as grossly apparent as they are in acute inflammation. Because of the fibroplasia and neovascularization, areas affected by chronic inflammation tend to be slightly swollen and firm. If fibrosis is extensive the lesions can be large and

disfiguring. Fibrosis (granulation tissue) is the best indicator that the inflammatory response is chronic.

Chronic inflammation tends to occur under the following conditions:

- Infections by organisms which are resistant to killing and clearing by the body tend to cause chronic inflammation. Such persistent organisms include some of the higher bacteria (including mycobacteria), fungi, and quite a few metazoan parasites.
- Repeated bouts of acute inflammation can result in a chronic reaction.
- Prolonged exposure to toxins can cause chronic inflammation.
- Chronic inflammation is a common component in many of the autoimmune diseases. Because the reaction is against a host epitope, which is always present, the inflammation is by definition chronic and persistent.[11]

INFLAMMATORY MEDIATORS

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury.[12] Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion.

	ACTION			
Mediator	Source	Vascular Leakage	Chemotaxis	Other
Histamine and serotonin	Mast cells, platelets	+	-	
Bradykinin	Plasma substrate	+	-	Pain
C3a	Plasma protein via liver	+	-	Opsonic fragment (C3b)
C5a	Macrophages	+	+	Leukocyte adhesion, activation
Prostaglandins	Mast cells, from membrane phospholipids	Potentiate other mediators	-	Vasodilatation, pain, fever
Leukotriene B_4	Leukocytes	-	+	Leukocyte adhesion, activation
Leukotrienes C_4 D_4 E_4	Leukocytes, mast cells	+	-	Bronchoconstriction, vasoconstriction
Platelet Activating Factor (PAF)	Leukocytes, mast cells	+	+	Bronchoconstriction, leukocyte priming
IL-1 and TNF	Macrophages, other	-	+	Acute-phase reactions, endothelial activation
Chemokines	Leukocytes, others	-	+	Leukocyte activation
	Macrophages, endothelium	+	+	Vasodilatation, cytotoxicity

Inflammation is the basic mechanism initiated by several stimuli, including mechanical and chemical injuries, and entities or events that invoke defense against microorganisms. It consist a cascade of cellular and microvascular reactions that serve to remove damaged and generate new tissue. However it often progress to painful or chronic harmful diseases requiring pharmacological treatment.[13]

CURRENT THERAPEUTIC APPROACHES

Co-administration of analgesic drugs is a popular therapeutic regimen for inflammatory diseases such as rheumatoid arthritis and osteoarthritis. An advantage of using combination therapy is that it can maximize the analgesic effects while minimizing the incidence of adverse side effects.[14] This goal is accomplished if the combination of inhibitors offers analgesic synergism, which allows a reduction in required dosage and decreases the incidence of undesired side effects.[15] For example, a current combination therapy includes nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, each of which has detrimental

side effects. The mechanism of action of NSAIDs involves inhibition of prostaglandin (PG) synthesis,[16,17] as well as direct central effects through modulation of neurotransmitter–receptor systems involved in pain transmission.[18-20] It is believed that the inhibition of cyclooxygenase-2 (COX-2) by NSAIDs underlies at least part of their anti-inflammatory and antinociceptive properties.[21] However, despite their ability to reduce inflammation and pain, NSAIDs cause a wide array of unwanted side effects such as renal failure, uncontrolled hypertension, aggravation of congestive heart failure, dyspepsia, and peptic ulceration hemorrhage and perforation.[22-25]

Table 1: list of anti-inflammatory drugs for inflammation and its side effects

- Aspirin (Anacin, Ascriptin, Bayer, Bufferin, Ecotrin, Excedrin)
- Choline and magnesium salicylates (CMT, Tricosal, Trilisate)
- Choline salicylate (Arthropan)
- Celecoxib (Celebrex)
- Diclofenac potassium (Cataflam)
- Diclofenac sodium (Voltaren, Voltaren XR)
- Diclofenac sodium with misoprostol (Arthrotec)
- Diflunisal (Dolobid)
- Etodolac (Lodine, Lodine XL)
- Fenoprofen calcium (Nalfon)
- Flurbiprofen (Ansaid)
- Ibuprofen (Advil, Motrin, Motrin IB, Nuprin)
- Indomethacin (Indocin, Indocin SR)
- Ketoprofen (Actron, Orudis, Orudis KT, Oruvail)

- Magnesium salicylate (Arthritab, Bayer Select, Doan's Pills, Magan, Mobidin, Mobogesic)
- Meclofenamate sodium (Meclomen)
- Mefenamic acid (Ponstel)
- Meloxicam (Mobic)
- Nabumetone (Relafen)
- Naproxen (Naprosyn, Naprelan)
- Naproxen sodium (Aleve, Anaprox)
- Oxaprozin (Daypro)
- Piroxicam (Feldene)
- Rofecoxib (Vioxx)
- Salsalate (Amigesic, Anaflex 750, Disalcid, Marthritic, Mono-Gesic, Salflex, Salsitab)
- Sodium salicylate (various generics)
- Sulindac (Clinoril)
- Tolmetin sodium (Tolectin)
- Valdecoxib (Bextra)

HERBAL DRUGS

Various herbal medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge about their mechanisms of action is known.[26] Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy.[27]

In many industrialized countries herbal medicines are gaining popularity as alternative and complimentary therapies. Some of the plants are used as food or medicine.[28] These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic and anti-microbial functions.

It is unambiguous that the survival and enhanced life span of human beings is made possible by the medicinal plants. The dependence of early man on medicinal plants is as old as the civilization itself. The therapeutic property of the medicinal plants is the outcome of the active constituents; these pharmacologically active constituents were synthesized and stored in different plant parts. Researchers are trying to explore this treasure of bio active molecules to convert the natural chemicals in a form useful for modern systems of medicine. The chemical constituents of herbal drugs were believed to have better compatibility with the human body and hence less side effects associated with them. Hence there is a growing trend in screening new herbs with subsequent isolation of the bioactive molecules from them. The traditional medicines have been derived from rich traditions of ancient civilizations and heritage. Indigenous systems of medicine across the world have enriched the present knowledge about the secondary metabolites and hence much of the scientific investigations are associated and relay on traditional systems of medicine. Indian system of medicine is considered as one of the richest ethnobotanical source and the work presented herein is a study carried out on a plant frequently used in Ayurvedic system of medicine.

RICE

Rice (Oryza sativa L.) is one of the most important staple food crops. About 3 billion people, nearly half the world's population, depend on rice for survival. In Asia as a whole, much of the population consumes rice in every meal. In many countries, rice accounts for more than 70% of human caloric intake.[29]

Rice is considered susceptible to salinity particularly during early vegetative and later at the reproductive stages.[30,31] Rice genotypes vary considerably in salinity tolerance that is principally due to additive gene effects.[32] One major approach to generate salt-tolerant rice cultivars through breeding is to maximize the genetic diversity between parental genotypes that is usually estimated by measurements of morphological and physiological differences. This conventional approach for screening rice genotypes can be costly, space-

and time-consuming and labor-intensive, and requires large sample size. Moreover, salt tolerance among genotypes can be altered by other environmental factors other than salinity such as temperature, light or humidity.[33] Identifying genetic variations of different salt tolerance genotypes based on DNA polymorphism offers several advantages over measuring physiological traits. Evaluation of genetic diversity using DNA marker technology is non-destructive, requires small amount of samples, is not affected by environmental factors, and does not require large experimental setup and equipments for measuring physiological parameters.

THERAPEUTIC ACTIONS OF RICE

Complementary and alternative medicine (CAM) regroups diagnostic and therapeutic approaches not included within allopathic medicine. Among the various CAM, "herbal medicine" is the most popular and fastest growing approach used to treat various ailments worldwide. "Njavara" (Oryza sativa L., variety "njavara") is a unique, indigenous, medicinal rice variety that matures in about 70 days' time and is traditionally used in Ayurvedic system of medicine practiced in Kerala, India. Several reports are available on the agronomic evaluation; lipid profile; thermal, starch and genetic characteristics of Njavara grains.[34-37] In short, the available scientific reports show that there are no detailed reports on the medicinal activity of authentic samples of Njavara. Recently they had reported the antioxidant, anti-inflammatory and chemical indices of "black glumed" Njavara, compared with staple, pigmented (Palakkadan Matta) and white (Sujatha) varieties.[38,39]

2. REVIEW OF LITERATURE

- **Jeong K (2016)**[40] examined the physiology and molecular regulation of phosphorus (P) remobilisation from vegetative tissues to grains during grain filling is poorly understood, despite the pivotal role it plays in the global P cycle. To test the hypothesis that a subset of genes involved in the P starvation response are involved in remobilisation of P from flag leaves to developing grains, we conducted an RNA-seq analysis of rice flag leaves during the pre-remobilisation phase (6 DAA) and when the leaves were acting as a P source (15 DAA). Several genes that respond to phosphate starvation, including three purple acid phosphatases (OsPAP3, OsPAP9b and OsPAP10a), were significantly upregulated at 15 DAA, consistent with a role in remobilisation of P from flag leaves during grain filling. A number of genes that have not been implicated in the phosphate starvation response, OsPAP26, SPX-MFS1 (a putative P transporter) and SPX-MFS2, also showed expression profiles consistent with involvement in P remobilisation from senescing flag leaves. Metabolic pathway analysis using the KEGG system suggested plastid membrane lipid synthesis is a critical process during the P remobilisation phase. In particular, the upregulation of OsPLDz2 and OsSQD2 at 15 DAA.

- **Thitinan Kitisin (2015)**[41] analyzed the use of colored rice extracts as a new source of anti-oxidative and antiinflammatory effects, in Thailand. This study investigates the effects of different colored rice extracts in terms of their biological content, antioxidative activity, and their ability to reduce pro-inflammatory cytokines and matrix metalloproteinase (MMP) expression. Various colored rice from different rice cultivating areas in Thailand were used to obtain ethanolic extracts. The biological compounds in colored-rice extracts were determined by Folin-Ciocalteu colorimetric and pH-differential methods. To determine the anti-oxidative properties of colored-rice extract, DPPH radical scavenging, ferrous reducing power, and lipid peroxidation assays were used. The cytotoxicity of colored rice extracts was determined by MTT assay on a human promyelocytic leukemia (HL-60) cell line in vitro. The inhibition of pro-inflammatory cytokines (IL-6, TNF-α, NF-κB) and MMP expression in LPS-induced HL-60 cells was determined by ELISA assay. Moreover, MMP activity was determined by gelatinolytic zymography. The results found that red (Mun Poo, MP) rice exhibited high anti-oxidative activity and reduced pro-inflammatory cytokines

and MMP-2 expression in LPS-induced HL-60 cells. This study provides new insights into the potential use of Thai colored rice extracts, especially red rice, as a source of anti-oxidants and anti-inflammation.

- **Saba Shaikh (2016)**[42] investigated and compared the anti-inflammatory activity of an aqueous and methanolic extract of Hibiscus cannabinus (Malvaceae) using carrageenan-induced rat paw edema. The female spargue dawley rat weighing 150-180 g were randomly divided into 6 groups of 6 each. First group served as vehicle control, second group served as standard, third and fourth group served as high (400 mg/ kg) and low (200 mg/ kg) dose of methanolic extract of Hibiscus cannabinus leaves (MHCL) respectively and fifth and sixth group as high (400 mg/ kg) and low (200 mg/ kg) dose of aqueous extract of Hibiscus cannabinus leaves (AHCL) respectively. The In vivo anti-inflammatory activity was studied using carrageenan induce rat paw edema animal model. The estimation of liver and blood parameters consist of serum glutamic oxalate transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), lipid peroxidation (LPO), reduced glutathione (GSH) and superoxide dismutase (SOD). Both MHCL and AHCL extracts showed significant (*$p<0.05$) inhibition of rat paw edema in dose-dependent manner. The maximum percent inhibition in paw edema was found in MHCL at dose of 400 mg/ kg was 52.00% and AHCL at dose of 400 mg/ kg was 49.93%. Both MHCL and AHCL at dose of 400 mg/ kg reduce LPO level as 31.10 nmol/ g and 35.23 nmol/ g respectively when compared with standard indomethacin. An anti-inflammatory activity was found in both MHCL and AHCL extracts. But the MHCL showed more significant anti-inflammatory activity.

- **Annie George (2014)**[43] aimed to evaluate the anti-inflammatory activity of ethanolic and aqueous extracts of Polygonum minus (Huds) using in vitro and in vivo approaches. The in vitro tests used to evaluate ethanolic extract are cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), lipooxygenase (5-LOX), secretory phospholipase-A2 (sPLA2) inhibition assay whilst the in-vivo effect was measured by the ability of aqueous extracts to reduce paw edema induced by λ-carrageenan, in rats. The ethanolic extract inhibited the activities of 5-LOX and COX-1($p<0.05$) whilst the inhibitory effect on COX-2 was only moderate. A marked inhibition of 5-LOX was observed at 30 μg/ ml. The extract did not inhibit the activity of sPLA2.

The anti-inhibitory activity of the aqueous extract from this plant was evaluated using a rat model where inflammation was induced in the paws by injection of λ-carrageenan. The aqueous extracts from Polygonum minus administered at doses of 100 and 300 mg/kg body weight (b.w.), significantly (P.<0.01) reduced paw edema induced by λ-carrageenan in the experimental model, at 4 h compared to the vehicle control. Furthermore, administration of 100 mg/kg b.w. or 300 mg/kg b.w. completely reduced inflammation of the paw 4 h after injection. These findings suggest that aqueous extract of Polygonum minus possesses potent anti-inflammatory activities.

- **Mrutyunjay M Mirje (2014)**[44] studied that, inflammation continues to be an area of great interest for research, probably due to the non availability of a safer and more effective anti-inflammatory agent. This has led to increase in demand for natural products with anti-inflammatory activity having fewer side effects. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are used in the treatment of inflammation, fever and pain. However, NSAIDs cause gastric damage as a major adverse reaction. In this study, the antiinflammatory activity of O. sanctum alone and in combination with indomethacin was studied using Carrageenan-induced rat paw edema. Aqueous extract of O.sanctum (200mg/kg or 400mg/kg) was administered alone and in combination with indomethacin (25mg/kg) to separate group of rats and paw volume was measured by plethysmometer and compared with control group. All the test groups showed significant (P<0.05) anti-inflammatory effect in Carrageenan-induced rat paw edema. The reduction of edema by O. sanctum was better than that of the standard anti-inflammatory drug, indomethacin and on co-administration marginally improved the anti-inflammatory profile of indomethacin. O.sanctum possesses significant anti-inflammatory activity probably due to inhibition of both cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism (dual inhibitory property).

- **Shrinivas Sharma (2009)**[45] investigated the anti-inflammatory properties of aqueous extract of the leaves of H. integrifolia, Planch. The hind paw edema was produced in rats by subplanter injection of carageenan. The aqueous extract of H. integrifolia, Planch. (AHI) at dose (250 and 500 mg/kg p.o) was given to observe % inhibition of paw edema which were comparable with indomethacin (10 mg/kg p.o)

used as a reference drug. The extract administered orally at doses of 250 and 500 mglkg p.o produced a significant ($P < 0.05$) dose dependent inhibition of edema formation. A significant % inhibition of paw edema by the aqueous extract of leaves of H. integrifolia, Planch. and its almost nearby same % inhibition with indomethacin suggest its usefulness as an anti-inflammatory agent.

- **Sreena K (2016)**[46] evaluated the anti-inflammatory property of the methanolic extract of the whole plant of Smithia sensitive (MESS) by both in vitro and in vivo method. Smithia sensitiva belonging to the family Fabaceae is a plant used as an anti inflammatory and anti oxidant drug by tribal peoples in kerala. Smithia sensitiva is a low growing annual herb 30-90cm long and it is distributed widely in Hilly areas. The whole plant is traditionally used as Refrigerant, Galactogogue and as lotion in headaches. In vitro method was estimated by bovine serum albumin denaturation (BSA) method and in vivo method was estimated by Cotton pellet-induced granuloma method. Both the methods showed significant anti-inflammatory property of the methanolic extract. The MESS at a concentration of 400µg/ml showed potent activity on comparing with the standard drug.

- **Youmbie Djanche Duplex Bonheur (2015)**[47] suggested that the etiology of inflammation can partly be explained by oxidative stress, this study was directed to evaluate the anti-inflammatory and antioxidant effects of the stem bark aqueous extract of Rauwolfia vomitoria in female Wistar rats. In sub-chronic study, cotton pellet and carrageenan induced granuloma as well as formalin induced paw oedema models were used to evaluate the anti-inflammatory potential of the plant extract. For the chronic model, we assessed the effect of the extract against Complete Freund's Adjuvant (CFA) induced inflammation. Administration of the aqueous extract (300 mg/kg) significantly reduced the formation of humid (31.25%) and dry (28.49%) granuloma in cotton pellet method compared with control. At the same dose, the plant extract also exhibited a significant sub-chronic anti-inflammatory effect by decreasing exudates volume (38.46%) and leukocytes number (53.68%) in air pouch test, and by reducing the paw oedema (49.60%) induced by formalin. Daily oral administration of the extract (300 mg/kg) or dexamethasone (1 mg/kg) for 10 and 12 days significantly inhibited by 55.98% and by 74.41% the paw eodema induced by formalin and CFA respectively when compared to control groups. CFA used alone significantly

decreased reduced glutathione (GSH) level as well as superoxide dismutase (SOD) and catalase (CAT) activities in liver, kidney and spleen when compared to normal rats. Treatment with Rauwolfia vomitoria extract and dexamethasone significantly restored GSH, SOD and CAT in all investigated tissues. In addition, the plant extract significantly restored malondialdehyde (MDA), protein and nitrite concentrations in serum and tissues (liver, spleen and kidney) by reducing their content, as compared to CFA control group. Keeping in this view, the data suggest that Rauwolfia vomitoria aqueous extract may possess antiinflammatory and antioxidant effects.

- **Kola Phani Kumar (2014)**[48] aimed for scientific evaluation of the anti-inflammatory activity of aqueous extract of Gliricidia sepium linn. (fabaceae) flowers by in-vitro and in vivo models. The anti-inflammtory activity of aqueous extract obtained by decoction was evaluated by in vitro HRBC membrane stabilization assay and in vivo carrageenan induced paw edema model in albino wistar rats. Aqueous extract showed dose dependant anti-inflammatory activity in human red blood cell membarane stabilization method at different concentrations (100-500 µg/kg) with a percentage protection of 7.15, 11.25, 22.71, 24.83 and 26.95 compared to standard diclofenac 32.09 % at 10 µg/kg. Diclofenac sodium at 10 mg/kg, aqueous extract administered at a dose of 250 and 500 mg/kg p.o. at 1, 3, 6 and 8 hours significantly ($p<0.05$) decreased and increased the volume of paw edema & % protection compared to carrageenan group and dicclofenac, respectively. The aqueous extract has shown a significant ($p<0.05$, $p<0.01$, $p<0.001$) percentage inhibition of paw edema 69.81±2.93 and 78.07±3.19 on 8th hour at 250 and 500 mg/kg, respectively. These results provide a scientific basis for the use of the flowers of Gliricidia sepium as an anti-inflammatory agent.

- **Murugesan P (2012)**[49] found the effect of aqueous and ethanolic extracts of bark of Ficus religiosa for its anti-inflammatory activity in Hamsters. Anti-inflammatory activity was evaluated using acute inflammatory models like carrageenan induced paw edema models. The biochemical parameters like thiobarbituric acid reactive substances (TBARS), enzymatic anti-oxidants and non enzymatic anti-oxidants were carried out in blood and tissues of control and experimental animals in each group. Oral administration of the ethanolic extract (Ficus religiosa bark) at the dose 100mg/kg body weight (b.w) showed significant effect than aqueous extract and was

also much comparable to that of standard drug, Ibuprofen. The mechanism of anti-inflammatory effect of Ficus religiosa is probably due to their inhibitory action on the release of mediators of inflammation.

- **Habibur Rahman (2015)**[50] analyzed the in-vitro anti-inflammatory and anti-arthritic activity of Joha Rice, an aromatic indigenous rice of Assam, India. The ethanolic extract of Oryza sativa Var. Joha rice (EEOS-JR) was studied for in-vitro anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method and In-Vitro anti-arthritic activity by bovine serum protein denaturation method and egg albumin denaturation method. The activity of ethanolic extract of Joha rice was compared with standard antiinflammatory drug Diclofenec. It is found that EEOS-JR at concentration of 100, 250 and 500 mcg/ml showed 51.12, 58. 75 and 63.77% protection of HRBC in hypotonic solution respectably, whereas, standard diclofenac at 50, 100 and 250 mcg/ml which showed 68.11, 73.83 and 76.17% protection of HRBC in hypotonic solution respectably. It also showed 39.29%, 52. 78% and 60.47% inhibition of denaturation @ 100, 250 and 500 mcg/ml of bovin serum whereas, standard diclofenac @ 100, 250 and 500 mcg/ml showed 93.20, 95.41 and 96.91% inhibition of denaturation of bovin serum. In egg albumin denaturation method at concentration of 100, 250 and 500 mcg/ml showed 75.00, 80.31 and 84.15% inhibition of egg albumin denaturation whereas, standard diclofenac 100, 250 and 500 mcg/ml which showed 27.78, 45.84 and 69.77% inhibition of egg albumin denaturation. It was found that ethanolic extract of Joha rice was more potent in inhibition of egg albumin denaturation than diclofenac. Finally, from results it can be concluded that Oryza sativa Var. Joha rice; an indigenous aromatic rice of Assam posses good in-vitro anti-inflammatory and anti-arthritic activities.

- **Shalini V (2012)**[3] summarized the effect of anti-oxidant and anti-inflammation with the preferential use of Njavara, a rice variety in indigenous medicine and the phytochemical investigations revealed the occurrence of a flavonoid, tricin at significantly higher levels compared to staple varieties. Flavonoids are a group of natural substances that are located in sources of vegetal origin and are able to regulate acute and chronic inflammatory responses. This study describes the new aspects of inflammatory suppression by the Njavara rice by evaluating the role of active constituent, tricin in the regulation of production of various pro- inflammatory

markers by human peripheral blood mononuclear cells stimulated with lipopolysaccharide. Treatment with tricin resulted in significant down- regulation of LPS-elicited production of TNF-α, IL-6, PGE2 and NO. Tricin was found to be a potential blocker of the expression of isoforms of nitric oxide synthase, cyclooxygenase and matrix metalloproteinases. Modulation of the cascade of molecular events in lipopolysaccharide signaling also includes inhibition of transcription factor NF-κB evidenced by the detection of enhanced p65 subunit in the nuclear extracts on tricin supplementation. The present study summarizes the role of the flavonoid, tricin in the modulation of the expression of different inflammatory mediators and revealed that the inhibitory effects on cell signaling pathways are responsible for its anti-inflammatory activity.

- **Settharaksa S (2014)**[51] concluded the effects of anti-inflammatory and antioxidant activity from cold press rice bran oil. For anti-inflammatory, their inhibitory activity of nitric oxide (NO) production using RAW267.4 cell lines was evaluated. And two methods for antioxidant activities, DPPH radical scavenging assay (DPPH assay) and Ferric Reducing Antioxidant Power (FRAP) assay were used and compared with gallic acid standard and ferric sulfate ($FeSO_4$), respectively. For the Oryza Sativa L. Khaw-khaw exhibited the highest activity against the NO production with an IC_{50} value of 41.96 μg/ml, followed by O. Sativa L. Hom Pathum (46.58 μg/ml), O. Sativa L. Hom Mali (53.84 μg/ml) and O. Sativa L. Hom Mali Gorkho (59.43 μg/ml). However, the antioxidant activity, DPPH method found O. Sativa L. Hom Mali Gorkho displayed the most potent effect with IC 50 value of 0.08 mg/ml, followed by O. Sativa L. Hom Pathum (0.11mg/ml), O. Sativa L. Hom Mali (0.12mg/ml) and O. Sativa L. Khaw khaw (0.88 mg/ml), respectively. The assay of FRAP showed the highest in O. Sativa L. Hom Mali Gorkho with an IC 50 value 2.27 mg/ml, followed by O. Sativa L. Hom Pathum (4.30 mg/ml), O. Sativa L. Khaw khaw (6.67 mg/ml) and O. Sativa L. Hom Mali (7.68 mg/ml), respectively. This study indicated that cold press rice bran oil from rice varieties in Thailand is responsible for anti-inflammatory and antioxidant activity. Therefore, this study supports the tradition use of cold press rice bran oil for treatment of inflammatory related diseases though the inhibition of nitric oxide release.

3. PLANT PROFILE[52,53]

KINGDOM	**:**Plantae
SUBKINGDOM	**:**Tracheobionta
DIVISION	**:**Magnoliophyta
CLASS	**:**Liliopsida
SUBCLASS	**:**Commelinidae
ORDER	**:**Cyperales
FAMILY	**:**Poaceae/Gramineae
GENUS	**:**Oryza
BOTANICAL NAME	**:**Oryza sativa L.

VERNACULAR NAME:

Languages	**-**	**Names**
Sanskrit	-	Dhanya, Vrihi, Nivara, Syali
Hindi	-	Dhan, Chaval
Marathi	-	Tandul, Bhat, Pendha
Bengal	-	Chal
Gujarat	-	Dangar, Choka
Tamil	-	Nellu, Arisi
Telugu	-	Biyam, Biyyam, Dhanyamu
Kannada	-	Akki, Bhatta
Malayalam	-	Ari, Navaranellu, Nellu

Oriya	-	Dhano
English	-	Rice, Paddy Rice, Chowdhury Rice

HABITAT:

Commonly in river valleys and other areas where water is abundant but also cultivated in some dry land areas.

DISTRIBUTION:

The plant is found throughout India; from Punjab to Bengal, Jammu to South India. Usually, the shrub or tree grows in dry deciduous forests of Central and Western India up to 1500 m on the hill slopes. It is widely found flora of Central and Western India. It is also found in Malay Peninsula, Java, Australia.

PLANT:

The rice plant is a member of Poaceae (old Gramineae) family. The common cultivated rice plant is an annual which usually grows to a height of a half meter or two meters but there are certain varieties that grow much taller (6-9 metres). Some deep water rice varieties grow with the gradual rise of the flood water level.

Rice plant can be divided into main two parts namely root system and shoot system:

ROOT SYSTEM:

When a rice grain germinates in a well drained, upland soil the sheath (coleorhizae) emerges. If it germinates in submerged low lands, coleoptile emerges ahead of the coleorhizae. The primary, embryonic roots (radicle) comes out through the coleorhiza shortly after it appears. This is followed by two or more secondary roots, all of which develop lateral roots. The embryonic roots later die and are replaced by secondary adventitious roots produced from the underground nodes of the culm.

SHOOT SYSTEM:

Collectively applies to all plant part visible above the ground level. It is mainly composed of culms, leaves and inflorescence (panicle).

Culm: The culm or stem is made up of a series of nodes and internodes. The rice culms are usually hollows except at the nodes. Each node bears a leaf and a bud. Under favorable conditions buds near ground level grow into tillers. The primary tillers give rise to secondary tillers which give rise to tertiary tillers.

Leaves: The leaves of rice are sessile in nature. They are borne at an angle, on the culm in two ranks along the stem, one at each node. The leaf blade is attached to the node by the leaf sheath. The rice leaf is similar to that of wheat, but is usually distinguished from it by the length of the ligule. In the rice, ligule is very prominent, usually more than one centimeter. The leaf number is more on a primary tiller than on the secondary and tertiary tillers.

Panicle: The rice inflorescence known as panicle is a group of spikelets borne on the uppermost node of the culm. The primary panicle branch is divided into secondary and sometimes tertiary branches. These bear the spikelet.

Spikelet: The individual spikelet consists of two outer glumes. All the parts found above the outer glumes are collectively called floret. It consists of a hard covering the two sections of which are known as lemma and palea (the glumes) and the complete flower is between them. The lemma and palea together are known as the "hull". The rice flower contains six functioning stamens (male organ) and a pistil (female organ). At the base of the flower are two transparent structures known as 'lodicules'. Rice is a self pollinated crop. When rice flower becomes ready to bloom, the lodicules become turgid and push the lemma and palea apart, thus allowing the stamens to emerge outside the open floret. Rupturing of the anthers then leads to the shedding of pollen grains. After the pollen grains are shed on stigma, the lemma and palea close.

Grain (Caryopsis): Rice grain develops after pollination and fertilization are completed. The grain is tightly enclosed by the lemma and palea. The dehulled rice grain is known as brown rice as brownish pericarp covers it. The pericarp is the outermost layer which envelopes the caryopsis and is removed when rice is milled and polished. The embryo lies at the ventral side of the spikelet next to the lemma. Adjacent to the embryo is a dot like structure the hilum. The embryo contains the plumule and radicle. The plumule is enclosed by a sheath known as coleoptile and the radical by the coleorhizae.

PARTS USED:

Leaves

CHEMICAL COMPOSITION:

The rice leaves contains alkaloids, saponins, terpenoids, steroids, flavanoids and phenolic compounds.

PROPERTIES AND USES:

The immense diversity of rice germ plasm is a rich source for many rice based products and is also used for treating many health related maladies such as indigestion, diabetes, arthritis, paralysis, epilepsy and give strength to pregnant and lactating mothers. Ancient Ayurvedic literature testifies the medicine land curative properties of different types of rice grown in India. Medicinal rice varieties like Kanthi Banko (Chhattisgarh), Meher, Saraiphul & Danwar (Orissa), Atikaya& Kari Bhatta (Karnataka), are very common in India. Few varieties cultivated in restricted pockets of Kerala for their medical properties e.g.Chennellu, Kunjinellu, Erumakkari & Karuthachembavu etc.

Oryza Sativa Leaves

4. AIM AND OBJECTIVE

The aim of the study is to evaluate the anti-inflammatory effect of leaves of *Oryza sativa* Linn. by carrying out the pharmacological studies with the aqueous extract of leaves of *Oryza sativa* Linn.

While over-the-counter painkillers can be really helpful, and in some cases may be the best option, they can also cause gastrointestinal upset and definitely aren't the best long-term strategy for coping with chronic pain. The goal is, of course, to try and help relieve pain by developing a new herbal remedy for the inflammatory action. Among the various Complementary and alternative medicine, "herbal medicine" is the most popular and fastest growing approach used to treat various ailments worldwide.[1]

The side effects produced by the synthetic agents include dizziness, weakness and increasing the gatric ulceration, elevates liver enzyme (diclofenac),nausea, vomiting, pruritis, headache, insomnia, steven johnson syndrome, peptic ulcer and nephrotoxicity caused by NSAIDs (aniline derivative), etc.

The key to reduce chronic inflammation in our body starts with our diet and being liberal in the use of high-quality herbs and spices is one simple way to boost the quality of our food. They are an inexpensive "secret weapon" that just about everyone can take advantage of. Spicing up our meals is not enough, however, if processed foods comprise the bulk part of our life. Thus there is a need to replace synthetic agents by safe and effective plant based herbal remedies as anti-inflammatory agents. Many plants extracts have been used as anti-inflammatory agent in folklore claim and in traditional medicines.

5. PLAN OF WORK

- Collection and authentication of the plant material.
- Preparation of extract.
- Preliminary phytochemical study of extract
- Pharmacological screening methods.
- *INVIVO* METHODS:
  - Carageenan induced paw oedema
  - Study on Biochemical parameters
    - In Serum
      - Total protein
      - Alkaline Phosphatase
      - SGOT (AST)
      - SGPT (ALT)
    - In Tissue Homogenate
      - LPO
      - GSH
      - SOD
      - CAT
      - GPx
      - Na^+/ K^+ ATPase

6. MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The leaves of *Oryza sativa* Linn. was collected during the month of December 2015 from Kumarapalayam, Tamilnadu, India and authenticated by Botanical Survey of India, Coimbatore (BSI/SRC/2/23/2014-2015/1192).

EXTRACTION

The green portions / fresh leaves attached to the plants were cut out from the root system of the plant and dried at room temperature for two weeks. They were then pulverized in a mortar and made into coarse powder for further analysis. Three hundred grams (300 g) of the pulverized sample was cold macerated successively in 750 ml of distilled water over 96 h period on a shaker (GFL D 3006 mgH, Germany) to ensure maximum extraction. These extracts were then filtered and concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). The aqueous extracts put in air tight containers stored in are frigerator. A yield of 11.8% (w/w) extract was obtained. The appearance of resulted extract was dark green color.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The aqueous extract of leaves of *Oryza sativa* Linn. was subjected to preliminary phytochemical screening[54,55,56].

1. Test for Alkaloids

The extracts was treated with diluted Hydrochloric acid and filtered. The filtrate was treated with various alkaloidal agents.

Mayer's Test: The extract was treated with Mayer's reagent, appearance of cream color indicated presence of alkaloids.

Dragendroff's Test: The extract was treated with Dragendroff`s reagent, appearance of reddish brown precipitate indicated presence of alkaloids.

Hager's Test: The extract was treated with Hanger's reagent, appearance of yellow colour indicated presence of alkaloids.

Wager's Test: The extract was treated with wager's reagent, appearance of brown precipitate was indicated presence of alkaloids

2. Test for Carbohydrates

The extracts were treated with 3 ml of alpha naphthol in alcohol and Conc.Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicated the presence of carbohydrates.

Fehling's Test: To the sample Fehling`s solution A and B was added and heated for two minutes. Appearance of reddish brown colour indicated presence of reducing sugars.

Benedict's Test: To the sample Benedict's solution was added and heated, appearance of reddish orange precipitate will indicate the presence of reducing sugars.

Barfoed's Test: The sample was treated with Barfoed`s reagent and heated, appearance of reddish orange precipitate was indicated presence of reducing sugars.

3. Test for Proteins

Biuret's Test: To the extracts Copper sulphate solution followed by Sodium hydroxide solution was added, a violet colour precipitates indicated presence of proteins.

Million's Test: To the extracts Million's reagent was added, appearance of pink colour indicated presence of proteins.

4. Test for Steroids

Libermann Burchard's Test: The extract was treated with Conc. Sulphuric acid and Glacial acetic acid followed by acetic anhydride, a violet ring appears at the junction of the liquids and appearance of green colour in the aqueous layer indicated presence of steroids.

5. Test for Sterols

The extracts was treated with 5%KOH solution, appearance of pink colour indicated the presence of sterols.

6. Test for Phenols

The extracts were treated with neutral Ferric chloride solution, appearance of violet colour indicated presence of phenols.

The extracts were treated with 10% Sodium chloride solution, appearance of creamcolour indicated presence of phenols.

7. Test for Tannins

The extract was treated with 10% Lead acetate solution appearance of white precipitate indicated presence of tannins.

The extracts were treated with aqueous bromine water; appearance of white precipitate indicated presence of tannins.

8. Test for Flavanoids

5ml of the extracts solution was hydrolyzed with 10%Sulphuric acid and cooled. it was then extracted with Diethyl ether and divided in to 3 portions in three separate test tubes.1ml of diluted sodium carbonate, 1 ml of 0.1 N Sodium hydroxide and 1 ml of diluted ammonia solutions was added to the first second and third test tube respectively. Development of yellow colour in each test tube indicated presence of flavanoids.

Shindoa's test: The extracts were dissolved in alcohol, to which a piece of Magnesium followed by drop wise addition of Conc. Hydrochloric acid and heated. Appearance of magenta colour indicated the presence of flavanoids.

9. Test for Gums and Mucilage

The extracts were treated with 25 ml absolute alcohol and then the solution will be filtered. The filtrate was examined for its swelling properties.

10. Test for Glycosides

A pinch of the extract was dissolved in Glacial acetic acid and few drops of Ferric chloride solution was added followed by the addition of Conc. Sulphuric acid, formation of red ring at the junction of the two liquids indicated presence of glycosides.

11. Test for Saponins

Foam test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes presence of saponins.

12. Test for Terpenes

The extracts were treated with tin and Thionyl chloride, appearance of pink colour indicated presence of terpenes.

PHARMACOLOGICAL STUDIES

ACUTE TOXICITY STUDIES [57]

Acute toxicity studies were performed by using OECD guide lines (Organization of Economic Cooperation and Development) 423. It's a step wise procedure with 3 female animals per step. Depending upon the mortality and/or morbidity status of animals, the average of 2-3 steps are necessary to allow judgment on the acute toxicity of the test substance. It results in the use of minimum number of animals while allowing for acceptable data based scientific conclusion. The procedure uses defined doses (2000 mg/kg body weight) and the result allows a test substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

EXPERIMENTAL PROCEDURE

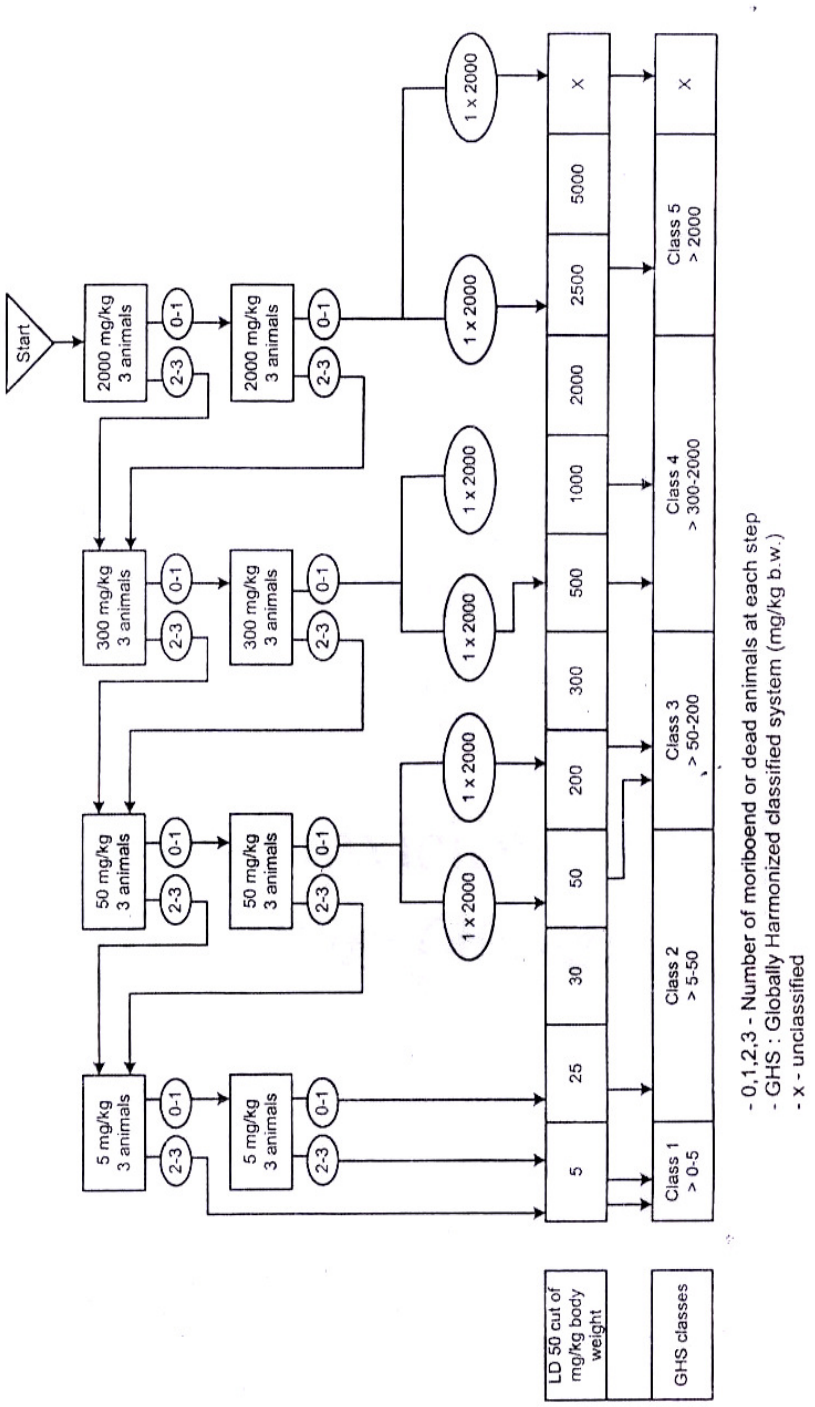
Three Male Wistar rats weighing 150-220 g body weight were used for the study, since the herbal extract are relatively non-toxic, 2000 mg/kg body weight was selected as the starting dose level of the extract (defined dose by OECD 423 guidelines). 18 hours prior to the administration of test drug, animals were fasted overnight with water *ad libitum*. Body weight of rats before and after administration of test drug noted and any changes in fur and skin and, eyes and mucous membrane, respiratory system and circulatory system were observed and also sign of convulsion, tremors, diarrhea, salivation, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

Behavioral profile: Alertness, irritability, restlessness and fearfulness.

Neurological profile: Spontaneous activity, reactivity, touches response and pain response.

Autonomic profile: Defecation and urination, lethality or death of animals was observed after 24 hours and 72 hours respectively.

FIG : OECD Guidelines for Acute Oral Toxicity



EXPERIMENTAL ANIMALS

Male Wistar rats weighing 200-250 g are used for animal studies, divided into group of 6 animals per cage. The animals were grouped in polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2°C) and relative humidity (50 ±5 %) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Bangalore, India) and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA (887/2PO/Re/S/2005/CPSCEA). The rats were acclimatized to laboratory condition for 14 days before commencement of experiment. The animals were fed with Gold Mohar commercial feed manufactured by Hindustan Lever Limited, Bangalore.

CHEMICALS

Carrageenan, Standard drug Indomethacin

METHODOLOGY

Group I: Served as control (received normal saline).

Group II: Rats were received 0.1 ml of 1 % carrageenan.

Group III: Rats were received indomethacin (10 mg/kg/p.o)

Group IV: Rats were received 1.0ml of aqueous extract of leaves of *Oryza sativa* Linn. (200 mg/kg/p.o) and 0.1 ml of carrageenan.

Group V: Rats were received 1.0ml of aqueous extract of leaves of *Oryza sativa* Linn. (400 mg/kg/p.o) and 0.1 ml of carrageenan.

CARRAGEENAN INDUCED INFLAMMATION

Acute inflammation or edema was induced by injection of 0.1 ml of carrageenan (1 % in 0.9 % sterile saline solution) into the rat's sub plantar surface of right hind paw region. The vehicle was administered 30 min. prior to injection of carrageenan and indomethacin was orally administered 1h prior to the injection of carrageenan. The pedal volume up to the ankle joint was measured using a digital plethysmometer at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} h.[3,58]

The percentage inhibition of edema volume between drug treated and carrageenan alone treated groups were calculated as follows,

$$\text{Percentage Inhibition} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc-Vt and Vc represented the mean increase in paw edema volume in control and drug treated groups.

Biochemical studies

Biochemical estimations were carried out in blood and tissues of experimental animals in each group.

Biochemical parameters in serum (collection of blood)

Blood was collected in clean dry test tubes and centrifuged at 3000 rpm for 5 min. Serum was separated and used for estimation of total proteins, alkaline phosphatase (ALP), aspartate transaminase (AST or SGOT), alanine transaminase (ALT or SGPT) by adopting standard methods.[59,60]

Biochemical parameters on tissue homogenate

After blood collection, the animals were sacrificed by cervical decapitation; the edematous tissue homogenate of rats, exposed to acute (carrageenan) inflammation model was collected and centrifuged at 600 x g for 10 min. The sediment, containing nuclei, unbroken cells and plasma membranes (nuclear fraction) were separated and the supernatant was subjected to centrifugation at 16,000 x g for 30 min. The sediment was suspended in 0.25 M sucrose buffer. Aliquots were withdrawn at 0 and 30 min intervals, immediately cooled at 0°C and centrifuged at 16,000 x g for 30 min. Lipid Peroxidation (LPO),glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPH), Na^+/ K^+ ATPaseactivity in the supernatant was then determined.[61,62-67]

Estimation of total protein (TP)[62]

The protein content of the tissue homogenate was measured by Lowry method. 0.5 ml of serum was mixed with 0.5ml of 10% TCA separately and centrifuged for 10 min. The precipitate obtained was dissolved in 1 ml of 0.1N NaOH, from this 0.1 ml used for the protein estimation.

Activity of Alkaline Phosphatase (ALP)[60]

Alkaline phosphatase activity was determined by Kind. 0.5 ml of buffered substrate (buffered substrate, pH 10.0) was added to control, blank and test and 1.5 ml of distilled water was added to all the tubes. Mix well and incubate for 3 min at 37°C. 0.05 ml of serum was only added to the test. Mix and incubate for 15 min at 37°C. 1 ml of colour reagent (Chromogen reagent) was added to all the tubes, 0.05 ml of serum was added to control tube. Mix well after the addition of each reagent and measure the optical density of Blank (B), Standard (S), Control (C) and Test (T) against purified water using a green filter (510 nm).

Estimation of SGOT (AST) [59]

The serum SGOT was estimated by the method Reitman and Frankel. 0.1 ml of serum was mixed with 0.5 ml of substrate reagent and incubated for 60 min at 37°C. 0.5 ml of colour reagent was added and further incubated for 20 min at 37°C. After the incubation, add 3 ml of alkaline reagent to stop the reaction and the colour intensity was read at 505 nm.

The SGOT activity was expressed as IU/L.

Assessment of SGPT (ALT)[59]

The serum SGPT was estimated by the method of Reitman and Frankel. 0.1 ml of serum was mixed with 0.5 ml of substrate reagent and incubated for 60 min at 37°C. 0.5 ml of colour reagent was added and further incubation for 20 min at 37°C. After the incubation, add 3 ml of alkaline reagent to stop the reaction and the colour intensity was read at 505 nm.

The SGPT activity was expressed as IU/L.

Estimation Lipid Peroxidation (LPO)[63]

Reagents: 1.5 ml of 8.1 % sodium dodecyl sulphate, 1.5 ml of 20 % acetate buffer (pH 3.5) and 1.5 ml of 0.8 % TBA solution. As a marker for lipid peroxidation the levels of Thiobarbituric acid reactive substances (TBARS) in the tissue was measured by the method of Ohkawa et al. A mixture of 0.4 ml of 10 % of tissue homogenate, 1.5 ml of 8.1 % sodium dodecyl sulphate (SDS), and 1.5 ml of 20 % acetate buffer (pH 3.5) and 1.5 ml of 0.8 % TBA solution was heated at 95°C for 1 h. After cooling, 5 ml of n-butanol-pyridine (15:1) was added, and the absorbance of the n-butanol- pyridine layer was measured at 532 nm.

Estimation of Glutathione (GSH)

Reagents: 10 % Trichloro acetic acid (TCA), 1.8 ml of EDTA, 0.6Mm 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) and 0.2M 4.0 ml of disodium hydrogen phosphate solution (pH 8.0). The GSH was determined by the method of Beutler and Kelly.[64] 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml of precipitating reagent TCA was added mixed thoroughly and kept for 5 min before centrifugation. To 2 ml of filtrate, 4.0 ml of disodium hydrogen phosphate solution and 1.0 ml of DTNB (5, 5-dithio bis 2-nitro benzoic acid) reagent were added and the absorbance was read at 412 nm.

Assay of Superoxide Dismutase (SOD)[65]

Reagents: 1.2 ml sodium pyrophosphate buffer (Ph 8.3, 0.025 ml/L), 0.1 ml phenazine methosulphate (186 mmol/L), 0.3 ml Nitro bluetetrazolium (300 nmol/L) and 0.2 ml NADH (780 mmol/L). The activity of SOD in tissue was assayed by the method of Kakkar. The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 ml/L), 0.1 ml phenazine methosulphate (186 mmol/L), 0.3 ml Nitro blue tetrazolium (300 nmol/L), 0.2 ml NADH (780 mmol/L) and diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml nbutanol. The chromogen in the butanol layer was measured at 560 nm against n-butanol.

Assay of Catalase (CAT)[66]

Reagents: Dichromate/ acetic acid reagent (5 % solution of potassium dichromate in acetic acid at 1:3 ratio), 0.01 M Phosphate buffer (pH 7.0), 0.2 M Hydrogen peroxide.

Catalase (CAT) was estimated by the method of Sinha (1972). The reaction mixture (1.5 ml) contained 1.0 ml of 0.01M phosphate buffer (pH 7.0) 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml dichromate acetic acid reagent (5 % potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was measured at 530 nm, CAT activity was expressed as µM of H_2O_2 consumed/ min/mg protein.

Assay of glutathione peroxidase (GPx)[67]

Reagents: 0.2 ml of glutathione, 0.1 ml of hydrogen peroxide and 0.5 ml of 10 % TCA. The glutathione peroxidase activity was assayed according to the method of Rotruk. To this, mixture, 0.2 ml of glutathione added followed by 0.1 ml of hydrogen peroxide. The contents were mixed well and incubated at 37°C for 10 min. along with the control tubes containing all the reagents except tissue homogenate. After 10 min, the reaction was arrested by the addition of 0.5 ml of 10 % TCA. The tubes were assayed for Glutathione content by activity was expressed as µmol/ mg protein.

Assay of Na^+/ K^+ ATPase[61]

Na^+/K^+ATPase were assayed according to the procedure of Bonting. The assay mixture contained 1 ml of the buffer, 0.2 ml of each KCl, NaCl, EDTA and ATP solution and 0.2 ml of the tissue homogenate. The contents were incubated at 37°C for 15 min. At the end of the incubation period the reaction was arrested by the addition of 1 ml of 10 % TCA. The tubes were centrifuged and the phosphorous content of the supernatant was estimated according to Fiske and Subbarow's method. The enzyme activity was expressed as micromoles of Pi liberated/h/mg of protein.

STATISTICAL ANALYSIS

The mean value ± SEM calculated for each parameter. Results were subjected to statistical analysis using ONE-WAY ANOVA, followed by Dunnet's t-test. The values were considered significant when $P< 0.001$, it was calculated using Graph pad prism Version 6.

7. RESULTS

PREPARATION OF PLANT EXTRACTS:

In a round bottom flask, the dried 300 g of aqueous extract of leaf powder of *Oryza sativa* Linn. was taken for extraction for 96 h. Amount of the extract of *Oryza sativa* Linn. obtained was 118.0 g. Percentage yield was found to be 11.8 % w/w.

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE EXTRACT OF *Oryza sativa* Linn. BARK:

The results of preliminary phytochemical analysis of the aqueous extract of leaves of *Oryza sativa* Linn. is shown in Table 1. The aqueous extract of leaves of *Oryza sativa* Linn. showed the presence of various phytochemical constituents such as

TABLE 1: List of Phytochemical Constituents Screened in *Oryza sativa Linn.*

S.No.	Constituents	Ethanolic Extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Protein	_
4.	Steroids	+
5.	Phenols	+
6.	Tannins	_
7.	Flavonoids	+
8.	Gums and Mucilage	_
9.	Glycosides	_
10.	Saponins	_
11.	Terpenoids	+
12.	Cardiac Glycosides	_

+ Present; **–** Absent;

ACUTE ORAL TOXICITY STUDY

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

TABLE 2: Acute Oral Toxicity Study of *Oryza sativa* Linn.

S. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before Test	After test				
1.	AEOS	2g/kg	160	165	No signs of toxicity	Nil	Nil	14 days
2.	AEOS	2g/kg	170	190	No signs of toxicity	Nil	Nil	14 days
3.	AEOS	2g/kg	200	225	No signs of toxicity	Nil	Nil	14 days

GROUPING OF ANIMALS

Animals are grouped into five categories which are as follows,

S.NO	GROUPS	TREATMENT	ROUTE
I	CONTROL	1.0 ml (Normal saline) – [7days]	p.o
II	CARRAGEENAN	0.1 ml (1% in 0.9% sterile saline solution) – [8th day]	i.p
III	INDOMETHACIN + CARRAGEENAN	10 mg/kg – [7 days + 8th day]	p.o
IV	AEOS 200 mg/kg + CARRAGEENAN	1.0 ml - [7 days + 8th day]	p.o
V	AEOS 400 mg/kg + CARRAGEENAN	1.0 ml - [7 days + 8th day]	p.o

IN-VIVO STUDY

MEASUREMENT OF PAW EDEMA

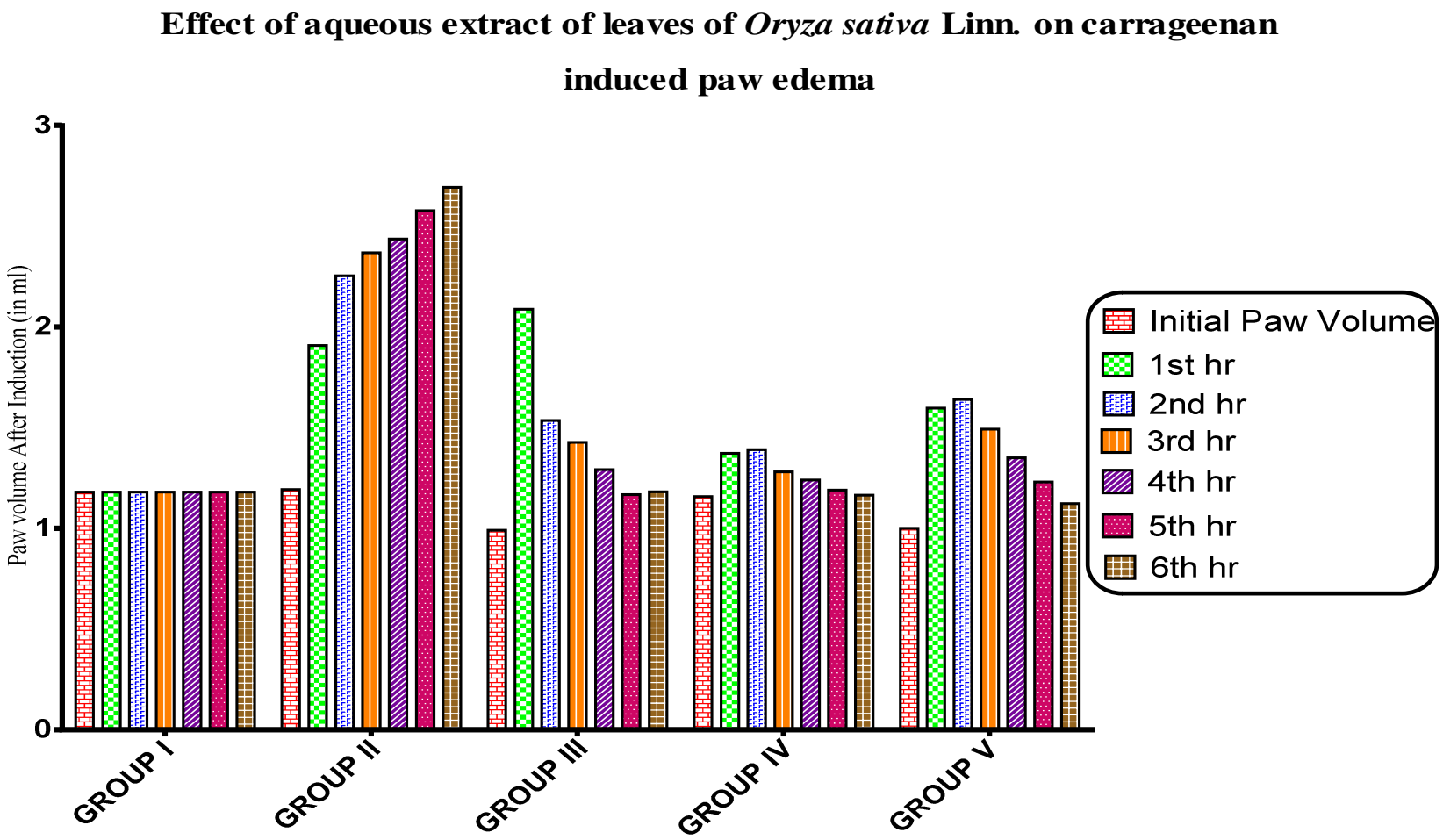
TABLE 3: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on carrageenan induced paw edema

Table 3 indicates that the change which occurs due to the treatment of aqueous extract of leaves of *Oryza sativa* Linn. in carrageenan induced paw edema by measuring the displacement value of mercury in plethysmometer.

Group	Initial Paw Volume	Paw volume After Induction(ml) as measured by mercury displacement at					
		1sthr	2ndhr	3rdhr	4thhr	5thhr	6thhr
I	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005
II	1.193 ± 0.007	1.908 ± 0.036****	2.253 ± 0.025****	2.368 ± 0.035****	2.437 ± 0.008****	2.577 ± 0.020****	2.692 ± 0.122****
III	0.990 ± 0.008	2.088 ± 0.033****	1.535 ± 0.023****	1.427 ± 0.022****	1.292 ± 0.061*	1.168 ± 0.006***	1.182 ± 0.006***
IV	1.157 ± 0.216	1.372 ± 0.026^{ns}	1.390 ± 0.157^{ns}	1.280 ± 0.051^{ns}	1.240 ± 0.040^{ns}	1.190 ± 0.026^{ns}	1.165 ± 0.007^{ns}
V	1.000 ± 0.057	1.597 ± 0.060**	1.640 ± 0.051***	1.492 ± 0.080**	1.350 ± 0.026***	1.230 ± 0.024*	1.123 ± 0.011^{ns}

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, ***p <0.001, **p<0.01, *p<0.1, ns-Non Significant).

FIG. 1: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on carrageenan induced paw edema



PERCENTAGE INHIBITION

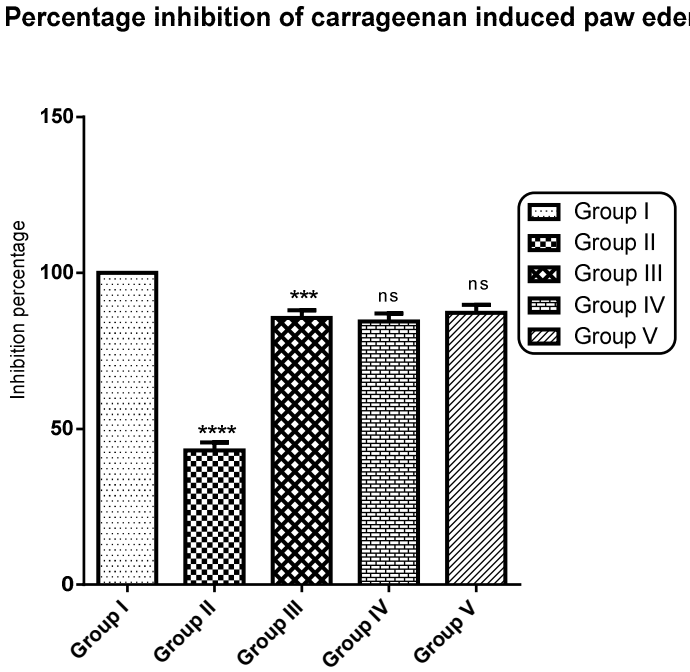
TABLE 4: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on percentage inhibition of carrageenan induced paw edema

Table 4 indicates that the change which occurs due to the treatment of aqueous extract of leaves of *Oryza sativa* Linn. in inhibition of carrageenan induced paw edema.

Group	Initial Paw Volume	6 hr (mm)	Difference in Paw	Inhibition percentage
I	1.180 ± 0.005	1.180 ± 0.005	0.00	100
II	1.193 ± 0.007	2.692 ± 0.122****	1.499	43.12
III	0.990 ± 0.008	1.182 ± 0.006***	0.192	85.57
IV	1.157 ± 0.216	1.165 ± 0.007^{ns}	0.008	84.42
V	1.000 ± 0.057	1.123 ± 0.011^{ns}	0.123	87.24

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, ***p <0.001, ns- Non Significant).

FIG. 2: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on percentage inhibition of carrageenan induced paw edema



a. BIOCHEMICAL PARAMETERS IN SERUM

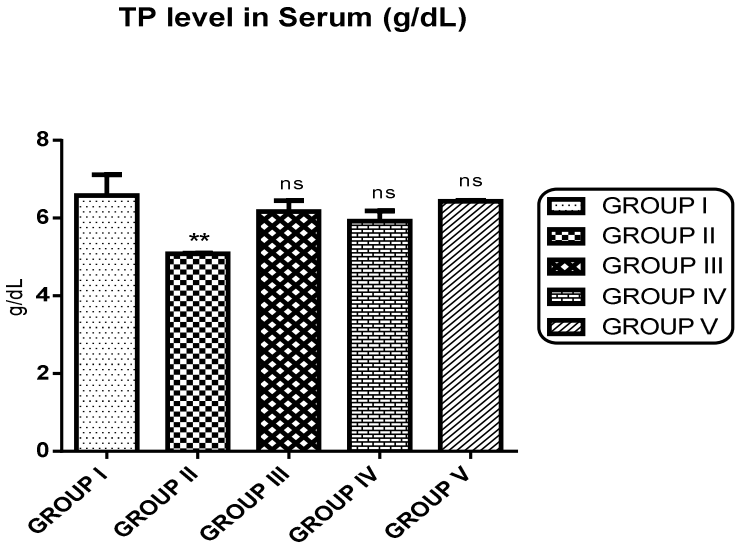
TOTAL PROTEIN (TP)

TABLE 5: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on Total Protein level in serum

Group	Treatment	Total protein (g/dL)
I	NORMAL SALINE	6.582 ± 0.539
II	CARRAGEENAN	5.085 ± 0.013**
III	INDOMETHACIN + CARRAGEENAN	6.173 ± 0.273ns
IV	AEOS 200 mg/kg + CARRAGEENAN	5.917 ± 0.274ns
V	AEOS 400 mg/kg + CARRAGEENAN	6.433 ± 0.027 ns

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (**p <0.01, ns- Non Significant).

FIG. 3: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on TP level in serum



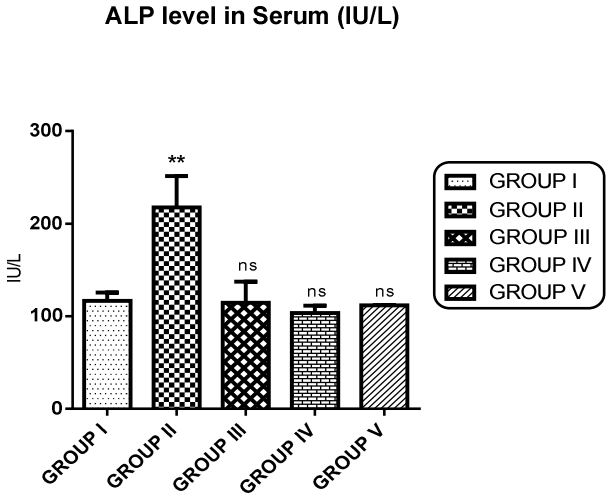
ALKALINE PHOSPHATASE (ALP)

TABLE 6: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on Alkaline Phosphatase level in serum

Group	Treatment	Alkaline Phosphatase (IU/L)
I	**NORMAL SALINE**	116.6 ± 8.953
II	**CARRAGEENAN**	217.2 ± 34.000**
III	**INDOMETHACIN + CARRAGEENAN**	114.4 ± 22.781ns
IV	**AEOS 200 mg/kg + CARRAGEENAN**	103.3 ± 8.171ns
V	**AEOS 400 mg/kg + CARRAGEENAN**	111.7 ± 0.361ns

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (**p <0.01, ns- Non Significant).

FIG. 4: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on ALP level in serum



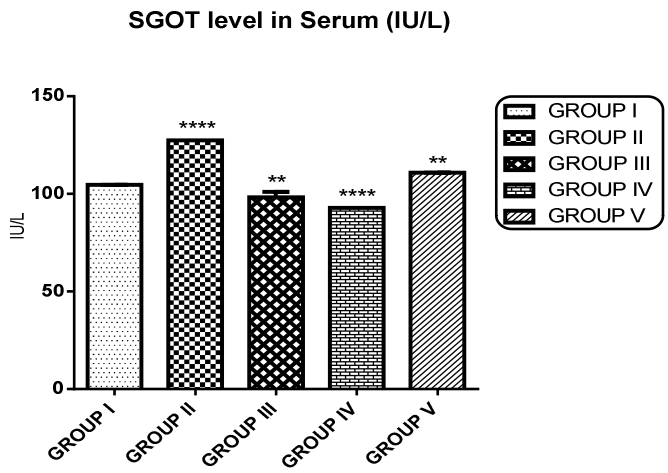
ASPARTATE TRANSAMINASE (AST OR SGOT)

TABLE 7: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on Aspartate Transaminase level in serum

Group	Treatment	Aspartate Transaminase (IU/L)
I	NORMAL SALINE	104.50 ± 0.060
II	CARRAGEENAN	127.30 ± 0.026****
III	INDOMETHACIN + CARRAGEENAN	98.19 ± 2.857**
IV	AEOS 200 mg/kg + CARRAGEENAN	92.87 ± 0.026****
V	AEOS 400 mg/kg + CARRAGEENAN	110.80 ± 0.020**

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, **p <0.01).

FIG. 5: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on SGOT level in serum



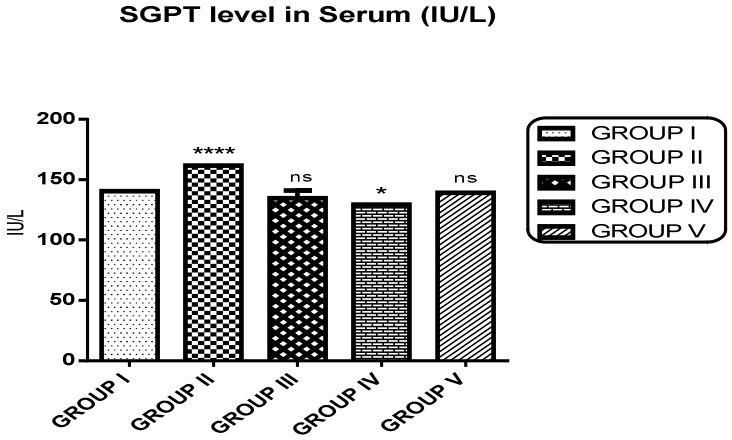
ALANINE TRANSAMINASE (ALT OR SGPT)

TABLE 8: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on Alanine Transaminase level in serum

Group	Treatment	Alanine Transaminase (IU/L)
I	NORMAL SALINE	140.4 ± 0.023
II	CARRAGEENAN	161.7 ± 0.057****
III	INDOMETHACIN + CARRAGEENAN	134.7 ± 6.265^{ns}
IV	AEOS 200 mg/kg + CARRAGEENAN	129.4 ± 0.007*
V	AEOS 400 mg/kg + CARRAGEENAN	139.1 ± 0.026^{ns}

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, *p <0.05, ns- Non Significant).

FIG. 6: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on SGPT level in serum



b. BIOCHEMICAL PARAMETERS IN TISSUE HOMOGENATE

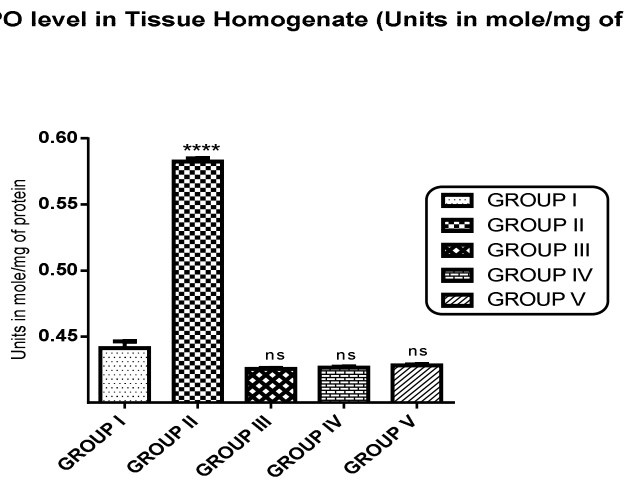
LIPID PEROXIDATION

TABLE 9: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on LPO level in tissue homogenate

Group	Treatment	LPO (Units in mol/mg protein)
I	NORMAL SALINE	0.441 ± 0.005
II	CARRAGEENAN	$0.582 \pm 0.002^{****}$
III	INDOMETHACIN + CARRAGEENAN	0.425 ± 0.0007^{ns}
IV	AEOS 200 mg/kg + CARRAGEENAN	0.426 ± 0.0007^{ns}
V	AEOS 400 mg/kg + CARRAGEENAN	0.428 ± 0.0007^{ns}

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V ($^{****}p < 0.0001$, $^{*}p < 0.05$, ns- Non Significant).

FIG. 7: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on LPO level in tissue homogenate



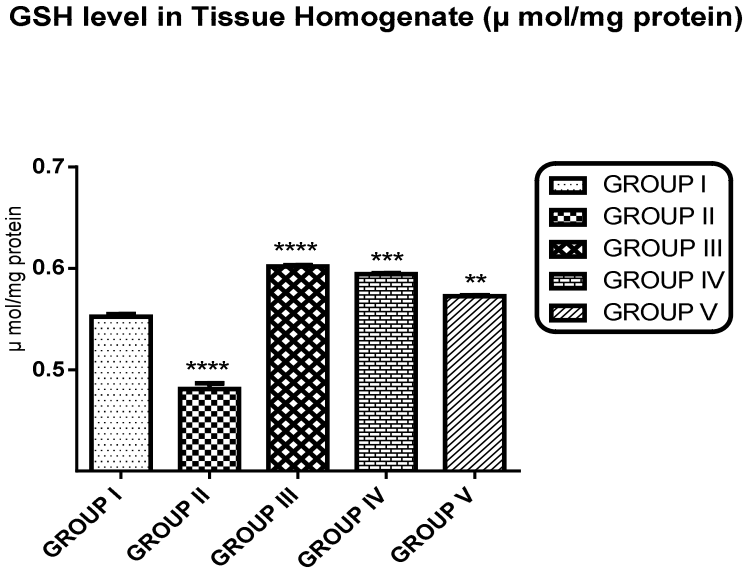
REDUCED GLUTATHIONE

TABLE 10: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on GSH level in tissue homogenate

Group	Treatment	GSH (µ mol/mg protein)
I	NORMAL SALINE	0.5523 ± 0.002
II	CARRAGEENAN	0.4810 ± 0.005****
III	INDOMETHACIN + CARRAGEENAN	0.6018 ± 0.001****
IV	AEOS 200 mg/kg + CARRAGEENAN	0.5943 ± 0.0008***
V	AEOS 400 mg/kg + CARRAGEENAN	0.5725 ± 0.0007**

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, ***p<0.001, **p <0.01).

FIG. 8: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on GSH level in tissue homogenate



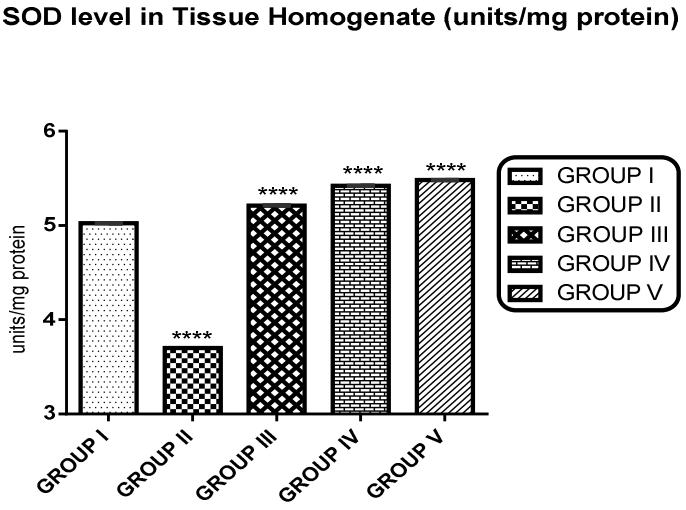
SUPEROXIDE DISMUTASE

TABLE 11: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on SOD level in tissue homogenate

Group	Treatment	SOD (units/mg protein)
I	NORMAL SALINE	5.023 ± 0.0007
II	CARRAGEENAN	3.702 ± 0.0005****
III	INDOMETHACIN + CARRAGEENAN	5.212 ± 0.0008****
IV	AEOS 200 mg/kg + CARRAGEENAN	5.421 ± 0.0008****
V	AEOS 400 mg/kg + CARRAGEENAN	5.483 ± 0.0007****

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (**** p <0.0001).

FIG. 9: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on SOD level in tissue homogenate



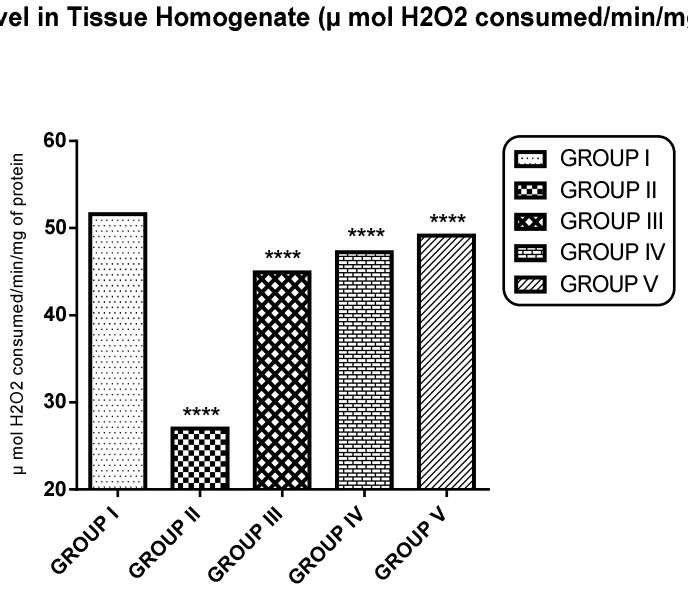
CATALASE

TABLE 12: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on CAT level in tissue homogenate

Group	Treatment	CAT (µ mol H_2O_2 consumed/min/mg of protein)
I	NORMAL SALINE	51.60 ± 0.001
II	CARRAGEENAN	27.01 ± 0.001****
III	INDOMETHACIN + CARRAGEENAN	44.91 ± 0.0009****
IV	AEOS 200 mg/kg + CARRAGEENAN	47.23 ± 0.0007****
V	AEOS 400 mg/kg + CARRAGEENAN	49.12 ± 0.0007****

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001).

FIG. 10: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on CAT level in tissue homogenate



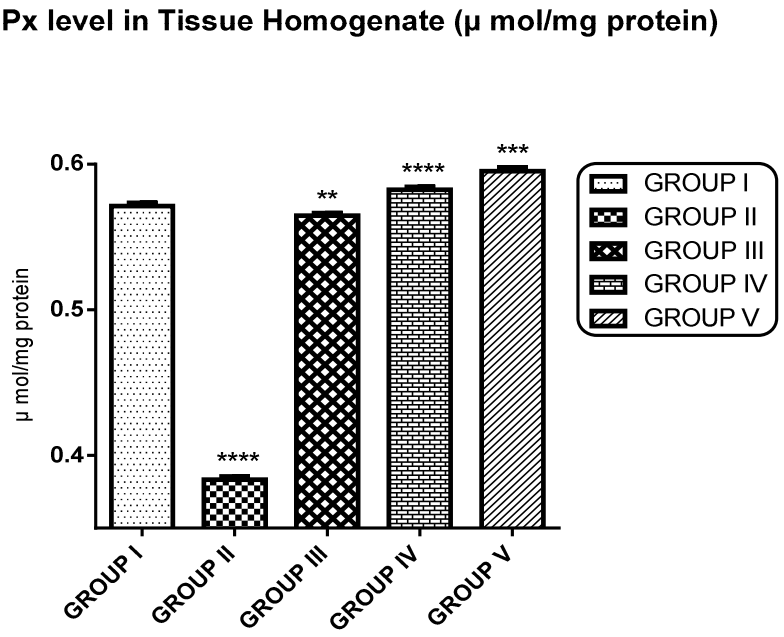
GLUTATHIONE PEROXIDASE

TABLE 13: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on GPx level in tissue homogenate

Group	Treatment	GPx
I	NORMAL SALINE	0.5713 ± 0.0008
II	CARRAGEENAN	0.3835 ± 0.0007****
III	INDOMETHACIN + CARRAGEENAN	0.5645 ± 0.0007**
IV	AEOS 200 mg/kg + CARRAGEENAN	0.5825 ± 0.0007****
V	AEOS 400 mg/kg + CARRAGEENAN	0.5952 ± 0.001***

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, ***p<0.001, **p <0.01).

FIG. 11: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on GPx level in tissue homogenate



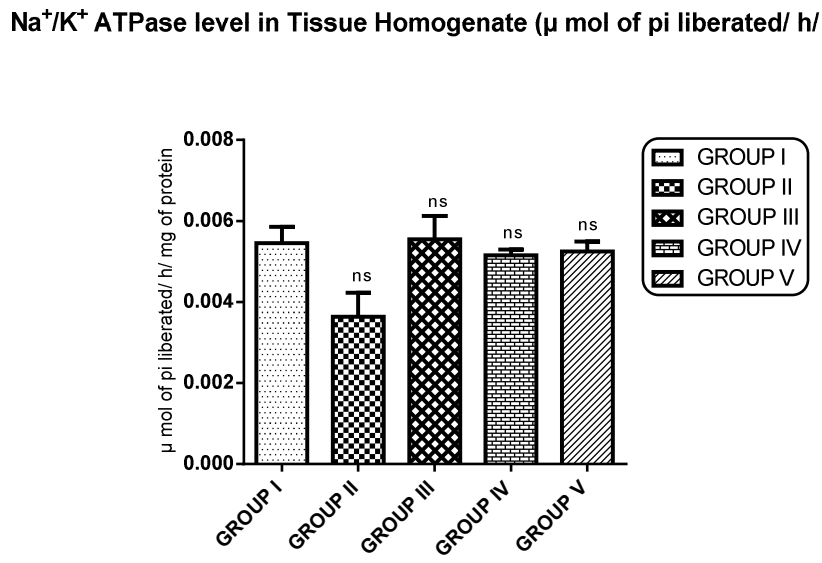
NA^+/K^+ ATPase

TABLE 14: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on Na^+/K^+ ATPase level in tissue homogenate

Group	Treatment	Na^+/K^+ ATPase (µ mol H2O2 consumed/min/mg of protein)
I	NORMAL SALINE	0.00545 ± 0.0003
II	CARRAGEENAN	0.00363 ± 0.0005^{ns}
III	INDOMETHACIN + CARRAGEENAN	0.00555 ± 0.0005^{ns}
IV	AEOS 200 mg/kg + CARRAGEENAN	0.00515 ± 0.0001^{ns}
V	AEOS 400 mg/kg + CARRAGEENAN	0.00525 ± 0.0002^{ns}

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III, IV, V (ns- Not Significant).

FIG. 12: Effect of aqueous extract of leaves of *Oryza sativa* Linn. on Na^+/K^+ ATPase level in tissue homogenate



8. DISCUSSION

At present, most inflammatory diseases are treated with conventional anti-inflammatory drugs, such as steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs). However, prolonged use of these drugs may produce many adverse effects, including gastrointestinal disorders,[68,69] immunodeficiency and humoral disturbances.[70] From long time plants have been used as source of drugs for the treatment of various ailments in developed as well as developing countries. In the present study, aqueous extract of leaves of *Oryza sativa* Linn. was evaluated for its and anti-inflammatory activity in experimental rats.

Preliminary phytochemical analysis of the aqueous extract of leaves of *Oryza sativa* Linn. showed the presence of phytochemical such as Alkaloids, Carbohydrate, Steroids, Phenols, Flavanoids, Terpenoids.

Acute oral toxicity studies of the aqueous extract of leaves of *Oryza sativa* Linn. were performed by using OECD 423 guidelines. Studies did not exhibit any lethality or any profound toxic reactions at a dose of 2000 mg/kg/p.o. According to the OECD 423 guidelines, for acute oral toxicity study, LD_{50} dose of 2000 mg/kg/p.o of the aqueous extract of leaves of *Oryza sativa* Linn. was found to be safe.

From this statistical analysis, it was concluded that the 'Carrageenan induced' Group II shows the inflammatory action and elevated level of Paw Volume is observed. But the 4^{th} and 5^{th} group deals with the anti-inflammatory activity of the aqueous extract of leaves of *Oryza sativa* Linn. which gives low Paw Volume at the end of 6^{th} h. The anti-inflammatory activity is more effective in Group IV Carrageenan induction with oral administration of aqueous extract of leaves of *Oryza sativa* Linn. of 200 mg/kg/p.o compared to Group V in which Carrageenan given after the oral administration of aqueous extract of leaves of *Oryza sativa* Linn. of 400 mg/kg/i.p. Significant inhibition of paw edema was observed with both doses tested till the sixth hour. However, maximum inhibition of paw edema was found to be in Group IV 87.24 % and although the inhibition of paw edema with the extract was higher than that found with the standard drug Indomethacin. The low percentage of inhibition is 43.12 % which belongs to the Group II i.e., Carrageenan alone induced. The duration of action was found to be comparable to that of Indomethacin till the sixth hour during investigation.

In the present study animals were treated with two doses of the aqueous extract of leaves of *Oryza sativa* Linn. (200 & 400 mg/kg), and negative control group is treated with Carageenan alone (0.1 ml). The blood serum was collected and estimated for the levels of TP, ALP, SGOT and SGPT. While compared to control group, ALP (116.6 ± 8.953; 217.2 ± 34.000**), SGOT (104.50 ± 0.060; 127.30 ± 0.026****), SGPT (140.4 ± 0.023; 161.7 ± 0.057****) were increased in negative control group respectively. TP level was decreased in negative control (5.085 ± 0.013**) when compared to the normal animals (6.582 ± 0.539). The extract treated groups when compared with standard (Indomethacin - 10 mg/kg) was almost equal to that of standard. In which, Group V (400 mg/kg) has slight increase when compared to Group IV (200 mg/kg) in the levels of TP (5.917 ± 0.274^{ns}; 6.433 ± 0.027 ^{ns}), ALP (103.3 ± 8.171^{ns}; 111.7 ± 0.361^{ns}), SGOT (92.87 ± 0.026****; 110.80 ± 0.020**), SGPT (129.4 ± 0.007*; 139.1 ± 0.026^{ns}) respectively.

A significant increase in the levels of lipid peroxides in tissue homogenate on administration of Carageenan (Group II) indicates the elevation of lipid peroxidation by free radicals. Due to increase in LPO (0.582 ± 0.002****), the other antioxidants GSH (0.4810 ± 0.005****), SOD (3.702 ± 0.0005****), CAT (27.01 ± 0.001****), GPx (0.3835 ± 0.0007****), and Na^+/K^+ATPase (0.00363 ± 0.0005^{ns}) levels were lowered significantly in the tissue homogenate of negative control group. Glutathione participates directly in destruction of hydrogen peroxide and also promotes the decrease in GSH, SOD, CAT, GPx, Na^+/K^+ATPase levels. The pretreatment with the aqueous extract of leaves of *Oryza sativa* Linn. (400 mg/kg) when compared to control group decreased the LPO (0.441 ± 0.005; 0.428 ± 0.0007^{ns}), CAT (51.60 ± 0.001; 49.12 ± 0.0007****), Na^+/K^+ATPase (0.00545 ± 0.0003; 0.00525 ± 0.0002^{ns}) levels and maintained GSH (0.5523 ± 0.002; 0.5725 ± 0.0007**), SOD (5.023 ± 0.0007; 5.483 ± 0.0007****), GPx (0.5713 ± 0.0008; 0.5952 ± 0.001***) levels in Tissue homogenate respectively.

The result obtained from the study indicates that the aqueous extracts of leaves of *Oryza sativa* Linn. (200 & 400 mg/kg) pretreatment possess significant protection against Carageenan induced paw edema. In that, Group V (AEOS 400 mg/kg) possesses more significant protection when compared with Group IV (AEOS 200 mg/kg).

9. CONCLUSION

Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities. These also decrease cardiovascular complications. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. The aqueous extract of leaves of *Oryza sativa* Linn. showed significantly higher inhibition percentage (stronger hydrogen-donating ability) positively correlated with total phenolic content.

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral, and anti-cancer activities. The present investigation suggests that the aqueous extract of leaves of *Oryza sativa* Linn. shows good antioxidant activity, reducing power, free radical scavenging activity and hepatic protection. Phytochemical screening of the aqueous extract of leaves of *Oryza sativa* Linn. reveals the presence of Alkaloids, Carbohydrate, Steroids, Phenols, Flavanoids, Terpenoids.

Acute oral toxicity studies of the aqueous extract of leaves of *Oryza sativa* Linn. did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w/p.o, in experimental rats.

Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation the results of this study are an indication that *Oryza sativa* Linn. can be effective in acute inflammatory disorders.[3] In this study, maximum inhibition of paw edema was found to be in Group IV pretreated with the aqueous extract of leaves of *Oryza sativa* Linn. (400 mg/kg). Although the inhibition of paw edema with the extract was higher than that found with the standard drug Indomethacin is suggestive of its protective effect against inflammation.

During inflammation, the extracellular proteins of the connective tissue can also be degraded by proteases released from (polymorphonuclear) PMNs or macrophages. It is not easily decided at present whether proteolytic degradation or free radical – dependent fragmentation is responsible for the phagocyte dependent connective tissue lesions and most likely these are not mutually exclusive. A preceding or simultaneous free radical induced fragmentation could render the connective tissue accessible to enzymatic

degradation. The aqueous extract of leaves of *Oryza sativa* Linn. (400 mg/kg) possess good anti-inflammatory activity against carageenan induced paw edema by increasing the level of TP, ALP, SGOT and SGPT in serum.

Our body has an effective defense system against free radical induced damage. It consists of a set of endogenous antioxidant enzymes, two of the key components of which is catalase (CAT) Superoxide dismutase (SOD).[71] Superoxide production by leukocytes apparently contributes to the killing of ingested bacteria.[72]

Regarding non-enzymic antioxidants, reduced glutathione (GSH) is a critical determinant of tissue susceptibility to oxidative damage. It is an intracellular reductant which is extensively found in cells. It protects cells against electrophilic attacks by xenobiotics such as free radicals peroxides. In the present study it is observed that, the antioxidant studies reveal that the levels of renal (hepatic enzymes) GSH, SOD, CAT, GPx and Na^+/K^+ATPase in the carageenan treated animals were decreased significantly along with an increased LPO content compared to control group. On treating with the aqueous extract of leaves of *Oryza sativa* Linn. (400 mg/kg) LPO, GSH, SOD, CAT, GPx, Na^+/K^+ATPase levels were increased with negative control.

In the present study it was confirmed that, the aqueous extract of leaves of *Oryza sativa* Linn. (400 mg/kg) possess anti-inflammatory activity. The result also supports the plant in the treatment of inflammation related disease traditionally.

Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of leaves of *Oryza sativa* Linn.

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