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"Pharmacological Evaluation of *Manilkara zapota(L.) P. Royen* fruit peel extract for Anti-inflammatory and analgesic effects in experimental animals"

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ABSTRACT

Background: Manilkara zapota (L.) P. Royen is commonly known as Chiku, belonging to the family Sapotaceae which is native to Mexico and central America and widely distributed in tropical and subtropical regions of Asia, Brazil and Australia. Manilkara zapota is a medicinal plant, various parts of this plant are traditionally used for treatment of several diseases, including analgesic, antipyretic, anti-diabetic, antioxidant, anti-inflammatory, and diuretic activity. The plant has been widely used in traditional systems of medicine in India.

Aim: Present investigation was undertaken aimed at "Pharmacological Evaluation of Manilkara zapota (L.) P. Royen fruit peel extract for Anti-inflammatory and analgesic effects in Experimental Animals."

Method: Ethanolic extract of Manilkara zapota fruit peel was subjected to continuous hot extraction by Soxhlet extraction process using ethanol (80%) as a solvent. Preliminary phytochemical evaluation of ethanolic extract was carried out for the determination of presence of phytoconstituents. The in-vitro Anti-inflammatory activity was evaluated by Heat induced hemolysis and Inhibition of albumin denaturation assay. and Antioxidant activity was evaluated by DPPH radical scavenging assay.

Result: The result suggested that the Phytochemical screening of ethanolic extract reveals the presence of alkaloid, flavonoid, carbohydrates, Tannin, phenol and saponin in Preliminary phytochemical evaluation. The in-vitro antioxidant activity revealed with the ethanolic extract of Manilkara zapota at the concentrations 50, 100, 150, 200 µg/mL exhibits 65%, 68%, 78%, 81% radical scavenging activity, whereas the Ascorbic acid as a standard drug at concentration 50, 100, 150, 200 µg/mL exhibit 71%, 79%, 84%, 89% radical scavenging activity respectively by using DPPH radical scavenging assay. In-vitro Anti-inflammatory activity reveales with the ethanolic extract of Manilkara zapota at concentration 50, 100, 150, 200 µg/ml exhibit 33%, 39%, 48%, 57% inhibition, whereas the Diclofenac as a standard drug at concentration 50, 100, 150, 200 µg/ml exhibit 45%, 54%, 69%, 78% inhibition of erythrocyte membrane repectively by using Heat induced hemolysis assay. While In-vitro Anti-inflammatory activity revealed with EEMZ at concentration 100, 200, 300, 400 µg/ml exhibit the 63%,66%,80%,85% inhibition respectively whereas Diclofenac as a standard drug at concentration 100, 200, 300, 400 µg/ml exhibit the 67%, 80%, 86%, 90% inhibition respectively by using Inhibition of albumin denaturation assay.

Conclusion : The study concluded that the antioxidant and Anti-inflammatory effects of Manilkara zapota Royen fruit peel extract that exhibit due to the presence of some phytoconstituents such as flavonoids, phenol, tannins, carbohydrates, alkaloids, saponin, carbohydrates ,amino acids as revealed in literature.

Keywords*: Manilkara zapota, Anti-inflammatory, Heat induced hemolysis.*

1. INTRODUCTION

Inflammation

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Definition

"Inflammation is the pathophysiological complex biological response of living mammalian tissues to harmful stimuli or injuries including pathogens, irritants or damaged cells that leads to the local accumulation of plasmatic fluid and blood cells.**"** (Amlan G. *et. al*.,2013) (S.Shukla *et. al*., 2009) .

It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation, however, if runs unchecked, led to onset of diseases such as vasomotor rhinnorrhoea, rheumatoid arthritis, atherosclerosis (Juvekar *et al*.2009). The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses. Therefore, development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary.(S. Shukla *et.al.,* 2009).

Inflammation is characterized by the cardinal symptoms of pain, heat, redness, swelling, loss of function .These symptoms mainly caused by direct effects of eicosanoids on the inflamed tissue. The rate- limiting steps in the synthesis of eicosanoids which includes prostaglandins (PG), leukotrienes (LT), and thromboxane (TX). Is the availability of arachidonic acid that is produced by the action of PLA_2. (Huwiler *et al.,* 2009).

Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever (Lucas *et al*., 2006).

Acute and chronic Inflammatory diseases remain one of the world's major health problems. It involves a complex array of biochemical process such as enzyme activation, inflammatory mediator release and extravasations of fluid,, cell migration, and tissue damage and repaire. (A.Alemu; W.Tamiru *et al.*,2018).

NSAIDs drug are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain., fever, inflammation and rheumatic disorders. However, their use is associated with adverse effects at the level of digestive tract ,ranging from dyspeptic symptoms, GIT erosions and peptic ulcer to more serious complication,such as over bleeding. (Md.H.Hossain; F.Jahan *et al*.,2012).

Inflammation is a complex pathological process mediated by a variety of signaling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α (Kinne *et al.,* 2000), that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site (White *et al*., 1999).

Inflammation diseases including, different typees of rheumatic diseases are the major problems associated with the presently available non-seriodal anti-inflammatory agents. The no. of plants derived drugs have been screened for their anti-inflammatory ,analgesic activity antioxidant activity.(S.H.Nile; Se Won park *et al*.,2013). Inflammation is a finely tuned, dyanamic process, and its dysregulation underlies many complex diseases (e.g.,sepsis, infectious diseases trauma, asthama, allergy, autoimmune disorders, transplant rejection, cancer, neurodegenerative diseases, obesity and atherosclerosis). Inflammatory processes are required for immune surveillance, optimal repair, and regeneratio after injury (Vodovotz *et al*., 2008).

Inflammation is an important cellular response triggered by various mechanical, chemical or immunological stress factors and it is regulated by a delicate balance between local factors that finally determine the outcome of the disease process: progression or resolution. . The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro- inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the off ending trigger. This is followed by an anti-inflammatory phase, in which resident tissue cells may acquire the potential for protecting themselves from further activation and injury (Huwiler *et al.,* 2009).

The NSAID are among the most commonly prescribed medication world wide. They consist of a group of drugs that are used in fever, pain, and inflammation because these drug posses antipyrectic, analgesic and anti-inflammatory properties Clinically ,tey are useful in relieving pain in many conditions, to arthritic pain. These drugs

are well known antiinflammatoy agents, and they exert their effects through the inhibition of prostaglandin synthesis by blocking the enzyme cyclooxigenase (COX).(Rebecca S.Y. Wong *et. al*.,2019).

Mechanism of action of inflammation

Mechanism of inflammation represents a chain of organized, dynamic responses including both vascular and cellular events with specific humoral secretions. These pathways involve changing physical location of white blood cells (monocytes, basophils, eosinophils, and neutrophils), plasma, and fluids at inflamed site. A group of mediators and other signaling molecules (e.g., histamine, prosta-glandins, leukotrienes, oxygen- and nitrogen-derived free radicals, and serotonin) are released by immune defense cells principally in the mechanism which can contribute in the event of inflammation.

Whatever, the inflammatory response is triggered by two phases: (a) acute and (b) chronic, and each is mediated by a different mechanism . These immune responses which involved in acute inflamma- tion can be divided into vascular and cellular inflammation. The responses which occur in microvasculature normally appear in few minutes following tissue injury or microbial infection in the presence of other inflammatory stimuli named vascular events. The occurrence of these processes is rapid and eventually will lead to vasodilation and subsequently makes the vessels become more permeable. This processes will result in entry of inflammatory mediators and produces interstitial edema (L.A.Abdulkhaleq et *al*.,2018).

Understanding inflammation has always been an enigma for mankind. something as minor as a bruise or something as major as a myocardial infraction can trigger this phenomenon. The major class of drug to suppress inflammation are nonsteroidal anti- inflammatory agent (NSAID) and corticosteroid but their toxic adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic Cushing's syndrome have limited their use. Looking at the present scenario, medicinal compounds derived from plants sources such as flavonoids, saponins, alkaloids, terpenoids, glycosides, coumarins could provide an excellent fountainhead to develop new anti-inflammatory agents, which could be more efficacious, safe, affordable, and accessible for patients.(G.G.,Meshram *et.al*.,2014).

Modulation of inflammatory reaction can be achived on various level targeting different cell types. first inflammatory cell including the monocytes / macrophages, neutrophiles, T- cells, eeosinophilies, etc. may be targeted. This includes; (1) inhibition of immune cell activation which leads to a reduced production and secretion of pro- inflammatory cytokines and chemokines. (2) reducing chemotaxis of immune cells by blocking the production, secretion or action of chemotactic factor (3) blocking the interaction of immune cell with vessel endotheial cells and therapy reducing the extravasation of immune cells into the inflamed tissue. (4) Blocking eicosanoid production in immune cells and tissue resident cells to reduce the cardinal symptoms of inflammation. (5) Activation of anti-inflammatory signaling cascade in immune cells and resident tissue cells to actively counteract and resolve an inflammatory reaction. (Huwiler *et al*., 2009).

It belived that current drug available such as Opioid and NSAID are not usefull in all cases of inflammatory disorders, because of their side effects and potency. As a result, a search for other alternative is necessary. Through medicinal plants, having wide variety of chemicals, novel anti-inflammatory agent could be discovered. Research on the biological activities of plants during the past two centuries has yielded numerous compounds for the development of modern drugs. (Juvekar *et al*.2009).

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A Spectrum of inflammatory response

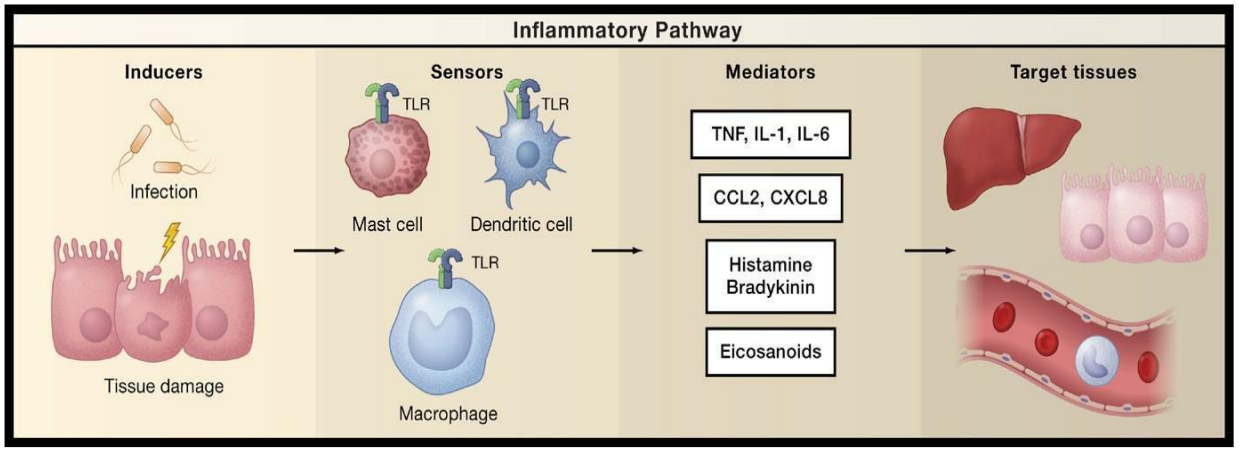

Fig: 1.1 Inflammatory pathway components

The inflammatory pathway it consists of inducers, sensors, mediators, and target tissues. Inducers initiate the inflammatory response and are detected by sen- sors. Sensors, such as Toll-like receptors (TLRs), are expressed on specialized sentinel cells, such as tissue- resident macrophages, dendritic cells, and mast cells. They induce the production of mediators, including cytokines, chemokines, bioactive amines, eicosanoids, and products of proteolytic cascades, such as bradykinin. These inflammatory mediators act on various target tissues to elicit changes in their functional states that optimize adaptation to the noxious condi- tion (e.g., infection or tissue injury) associated with the particular inducers that elicited the inflammatory response. The specific components shown represent only a small sample of the myriad different sensors, mediators, and target tissues involved in the inflammatory response.(Medzhitov Z.*et al*.,2010).

Analgesic

Definition

“ Pain is defined simply **“**as an unpleasant and emotional , experience associated with or without actual tissue damage**.”** (K.Kumari;H.Biswas *et al.*,2017). Pain and fever are being the most common complaints associated with inflammation.(A.A.Suralkar *et al*.,2012).

The pain sensation is described in many ways like sharp, pricking, electric, dull, chae, shooting, cutting, stabbing etc. Often it induces crying and fainting. It is produced by real or potential injury to the body. Pain may be acute or chronic. Acute pain is a sharp pain of short duration with easy identified cause. Chronic pain is the intermittent or constant pain with different intensities. It lasts for longer periods. It is somewhat difficult to treat chronic pain and it needs professional expert care. (K.Kumari; H.Biswas *et al.*,2017).

Pain sensation various parts of body is carried to brain by different pathway such as pathway from skin and deeper structure, pathway from face, pathway from viscera and pathway from pelvic region. Neurotransmitters involved in pain sensation are glutamate amd substance P are the neurotransmitters secreted by pain nerve endings. The A afferent fibers which transmit impulses of

fast pain secrete glutamate. C type fibres which transmit impulses of slow pain secrete substance P. (K.Kumari;H.Biswas *et al.*,2017).

Pain is in fact a very serious problem associated in 90% of diseases. NSAID'S, which are Used to treat pain ,have several side effects. There is a great need to develop some natural Agent (phytoconstituent), which are capable of treating both acute and chronic Pain (M.R.praddepkumar *et al*.,2015). Pain is in fact a very serious problem associated in 90% of diseases. NSAID'S, which are Used to treat pain ,have several side effects. There is a great need to develop some natural Agent (phytoconstituent), which are capable of treating both acute and chronic Pain (M.R.praddepkumar *et al*.,2015).

There are various form of analgesic that contribure to reducing pain. They are classified into three categories: **1** opioids analgesic (e.g. morphine ,codeine); **2**. non-opioid analgesics, such as non-steroidal anti-inflammatory drugs (NSAIds) among which stands out aspirin and diclofenac; **3**. adjuvant analgesics, which are compounds commonly administered for other reasons than pain, but can control it in certain situation. Opioids analgesic causes a maximal analgesia; they are considered broad spectrum drugs in the treatment of acute pain because of their great efficacy. (D.J. Marmitti; S.Bitencourt *et al*.,2016).
Analgesics relieve symptoms of pain but hardly affect its underlying cause. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. Again, plant-derived secondary metabolites have, over the years, greatly contributed to our current understanding of the important mechanisms related to the process of inflammation, pain transmission and treatment. Furthermore, they have permitted us to characterize receptor types and identify endogenous ligands involved in the mechanism of nociception. Plants, such as *Papaver somniferum*, *Cannabis sativa* and those of the Capsicum and Salix species, have greatly accounted for the development of clinically relevant drugs which are useful for the management of pain disorders (Hijazi Ali *et al.*, 2017).

Anti-oxidant

Free radical

Atom contain a nucleus, and electron move around the nucleus, usally in pair. A free radical is any atom or molecule contain one or more unpaired electron. The unpaired electron alter the reactivity of an atom or molecules, usally making it more reactive than the corresponding non-radical.(Halliwell B. *et al*.,1994).

"Antioxidant act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body**."** Antioxidant constituents of plant materials act as radical scavengers, and convert the radical to less reactive species(Mandal S. Yadav S.et al.,2009). Antioxidant can also protect the human body from free radicals and Reactive oxygen species effects.

Importance of herbal drug therapy

Fig: 1.2 Herbal drug

"A plant or part of plant or an extract or mixture of these used in herbal medicine .An herb is a plant part used

for its scent, flavor,and therapeutic properties .People used herbal medicine to try to maintain or improve their health or cure diseases. Digitalis is one of these examples and the number of these plants is not a lot. The mechanisms by which the herbs generally act are not established, however, most of medicinal plants possess antioxidant activities. The plants have been shown to effective by this property is various conditions including cancer, memory deficit and Alzheimer, atherosclerosis, diabetes and other cardiovascular diseases (Karimi Ali *et al*., 2015).

India is a rich source of medicinal plants and a number of plant derived oil.,extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only few of them hav been scientifically explored. Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China (Xia dynasty) and India (Vedic period) (Schuppan *et al*., 1999 / S. Shukla;A. Mehta *et al*.,2009) Herbal remedies are widely used in developing countries to manage pain and inflammation because of their cost, accessibility, and eco-friendly advantages.(A. Aleum *et al.*,2017)

Herbal therapy is a holistic therapy, integrating emotional, mental and spiritual levels. Life style, emotional, mental and spiritual considerations are part of any naturopathic approach. The use of herbs does not generally involve "drug" actions or adverse effects. Although medicinal plants are widely used and assumed to be safe, however, they can potentially be toxic (Karimi Ali *et al*., 2015).

World Health Organization (WHO) has set precise guidelines for the evaluation of the safety, efficacy, and quality of herbal medicines. They have inexpensive, easily and abundantly available, also with less side effects, low minium cost, complete accessesibility, more protection, potency and efficiency is very high.

Herbal drug is a chief constituent in traditional medicine and a common constituent in ayurvedic, homeopathic, naturopathic and other medicine systems. Herbs are usually considered as safe since they belong to natural sources . The use of herbal drugs due to toxicity and side effects of allopathic medicines, has led to rapid increase in the number of herbal drug manufacturers. For the past few decades, herbal drugs have been more and more consumed by the people with no prescription.(K. . Pathak; R. J. Das *et al*.,2013).

Antioxidant of the herbal medicines are also effective in reducing the toxicities of toxic agents or other drugs. Seed, leaves, stems, bark, roots, flowers, peels and extract of all of these have been used in herbal drugs. Herbal product have reached extensive adequacy as beneficial agents like antimicrobial, analgesic, sedative, anti-inflammatory, antidiabetic activities.(B. Maiti; Nagori B.P *et al*.,2011) These drugs have survived real world testing and thousands of years of human testing. Some drugs have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to counterbalance side effects. (K. Pathak; R. J. Das *et al*.,2013).

Herbal therapy, although still an unwrtitten science,is well esatablished in some countries and traditions and has become a way of life in alost 80% of population in rural areas. Chronic anti- inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. Their prolong use may causes severe adverse effects on chronic administration the most common being GIT bleeding and peptic ulcers. Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for self and effective anti-inflammatory and analgesic agents have been given priority in scientific research in herbal system of medicine. (M.Singh;V.Kumar *et al*., 2011) .

Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Manna *et al*., 2000). Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. Due to the known side effects of approved pharmaceuticals, patients often turn to alternative medicine which is considered "natural" and "healthy" Herbal medicine is thus gaining popularity, but lack of knowledge of the mechanisms and side effects of these preparations as well of safety regulations for their preparation may have serious consequences .(Boullata *et al.*, 2000).

Acute and chronic Inflammatory diseases remain one of the world's major health problems. It involves a complex array of biochemical process such as enzyme activation, inflammatory mediator release and

extravasations of fluid,, cell migration,and tissue damage and repaire.(A.Alemu; W.Tamiru *et al.*,2018). NSAIDs drug are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain., fever, inflammation and rheumatic disorders. However, their use is associated with adverse effects at the level of digestive tract ,ranging from dyspeptic symptoms, GIT erosions and peptic ulcer to more serious complication,such as over bleeding. (Md.H.Hossain; F.Jahan *et al*.,2012). Therefore to overcome the toxicity of NSAIDs, the development of new anti-inflammatory drugs is still necessary and the natural product such as medicinal plants could lead in discovering new anti-inflammatory drugs with less undesirable effects.

Now-a- days attention is being focused in the investigation of the efficacy of plat based drugs used in the traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies. (Md.H.Hossain; F.Jahan *et al*.,2012). Fruit and also peels are identified as rich source of antioxidant used to overcome oxidative stress.

The fact behind the health beneficial property of fruit is the large no. of phytoconstituents like phenolic ascorbic acid, carotenoids, gallic acid, saponine, terpene, tannin, flavonoid,sterols may have direct influence over the Radical scavenging potential.(K.Gomathy *et al*.,2013; P.Karle *et al*.,2019))

Advantages of Herbal Drug Therapy

- High low/ Minimum cost
- Complete accessibility
- Enhanced tolerance
- More protection
- Fewer side effects
- Potency and efficiency is veryhigh.
- Inexpensive
- Easily available
- Less side effect.

Importance of natural drug

Natural compounds are now gaining more pharmacological attention as many unexplored plant products are showing a wide range of activities like anti- inflammatory and anti-cancer. It is estimated that about 80% of the world's population primarily those of developing countries rely on plant-derived medicines for their healthcare needs. In many developed countries popular use of traditional/complementary and alternative medicine is also expanded due to great concern about the adverse effects of modern drugs.

It is estimated that approximately one quarter of the best selling drugs worldwide were natural products or derived from natural products. Nearly 25% of all prescribed drugs are derived from plants with or without further modification and still several pharmacologically active plant-derived compounds remain unexplored. The anti-inflammatory activities of plants are due to the secondary metabolites. These bioactive compounds consist of polyphenols, flavonoids, alkaloids, saponin ,tannin, terpenoids, steroids, carotenoids, coumarins and curcumins which is responsible for significant effects. (Rafieian *et al.,* 2012).

The majority of naturally occurring phenolics retain anti-oxidative and anti- inflammatory properties which appear to contribute to their chemo preventive or chemo protective activity. Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammatory activities are anticipated to exert chemo preventive effects on carcinogenesis, particularly in the promotion stage.

Examples are curcumin, a yellow pigment of turmeric (*Curcuma longa* L), the green tea polyphenol epigallocatechin gallate (EGCG) and resveratrol from grapes (*Vitis vinifera*) that strongly suppress tumor promotio. Thus, searching for inflammatory inhibitors with chemotherapeutic potential from natural sources is an alternative approach in the development of anti-inflammatory and anti-cancer drugs (Karimi *et al*., 2015).

Plant derived natural products such as flavonoids, tannin, phenol, terpenes and alkaloids have received considerable attention due to their diverse pharmacological properties including inflammatory, antipyretic and analgesic activities.(S.Shukla;A.Mehta *et al*.,2009) Plants contain numerous bioactive molecules that can improve the body's resistance to cellular stress and prevent the cytotoxicity of various agents (Newman *et al*., 2007).

Natural products and their derivatives have traditionally been the most common sources of drugs, and still represent a fairly large percentage of the pharmaceutical market. It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead structures for drug discovery (Shukla *et al*., 2010). Plants are a rich source of active ingredients for health care products, with many blockbuster drugs being directly or indirectly derived from plants (Newman et al., 2000). However, many high value plant- derived natural products remain undiscovered or unexplored for their pharmacological activity (Jurenka *et al.,* 2001).

II. REVIEW OF LITERATURE

2.1. General information of Manilkara zapota (L.) P. Royen Fruit Plant introduction:

Manilkara zapota (L.).P. Royen is large evergreen tree,more than 30m height with a dimeter upto 1.5m. Traditionally, almost part of plant used for its medicinal activity like anti- Inflammatory, anti-oxidant, cold, fever, pulmonary diseases, diarrhea, analgesic, antimicrobial. Tree medium to large tree with a pyramidal to rounded canopy with many branches. In which Young branches are horizontally and dropping. A milky latex knows as "chicle" exudes from all tree parts. These long-lived trees grow slowely but after many years, may reach 20-30 min height .Branches are horizontal or dropping .(Kamaranga P.*et al.*2007).

Manilkara zapota (L.)P. Royen is a popular fruit crop, species from the *Sapotaceae* family, which is widely cultivated in most tropical reasons across the world. Different plant parts such as latex, fruit ,leaf ,bark ,seed and timber are being used for various purposes. The fruit is nearly oval, round, oblate, conical or ellipsoidal; unripe fruit is rigid, gummy and very astringent, smooth-skinned coated with a sandy brown scurf until fully ripen.(Karle P.*et al* 2019*).*

The peel of *Manilkara zapota* is rich in antioxidant principles, containing many important bioactive phenolic compounds known to provide its health benefits. In particular, a rich variety of phenolic compounds (as sources of natural antioxidants) and flavonoids present in it have attracted the attention of many researchers and practitioners. Among these, flavonoids are capable of effectively scavenging reactive oxygen species and becomes a strong antioxidant due to presence of phenolic hydroxyl groups. Most of the flavonoids are already reported anti- diabetic, anti- inflammatory, anti-allergic and antiplatelet agents **(**Karle P.*et al* 2019).

Plant profile:

Manilkara zapota (L.) P. Royen fruit also known as sapodilla belongs to the Sapotacea family which having an ecologically diverse family of 700 species and up to 40 ill- defined genera, pamtropically distributed. (Karle P.*et al 2019)*

Fig. 2.1 *Manilkara Zapota (L.) P. Royen* fruit variety with Round and Elongated Nomenclature (Taxonomical classification)

Table No: 2.1 Classification of Genus *Manilkara zapota* is as follows (Karle P.*et al* 2019).

Kingdom	Plantae (plants)
Sub-kingdom	Tracheobionta (vascular plants)
Super division	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledanae)
Sub-class	Dilleniidae

Order	Ebenales
Family	Sapotaceae
Genus	*Manilkara* Adans. (manilkara) malabar
Species	*Manilkara zapota* (L.) van Royen

Table No: 2.2.Vernacular name or common name of Manilkara Zapota fruit (Karle P.*et al* 2019,Agroforestry Database 4.0*(orwa et al.*2009).

COUNTRY	VERNACULAR / COMMON NAME
India	Chikoo, Chicku, Chiku
Mexico	Chicopote,Chicozapote
Puerto Rico	Nispero
English	Sapodilla
West Indies	Nasebery
Thailand	Lamoot, Lamut
Malaysia	Chikoo
Bahamas	Dilly
Brazil	Sapoti, Sapotilha
Cuba	Sapota ,Sapote
Spanish	Zapotillo (small zapot), nispero
Creole	Sapoti

Synonyms (Karle P.*et al* 2019)

- Achras sapota L.
- Achras zapota L. var. zapotilla Jacq.
- Achras zapotilla Nutt.
- Achras mammosa L.
- Manilkara achras (Miller) Fosberg
- Manilkara zapotilla (Jacq.)GillySapota
- zapotilla (Jacq.) Coville
- Sapota achras Miller
- Sapota zapotilla (Coville) (Karle P.*et al* 2019).

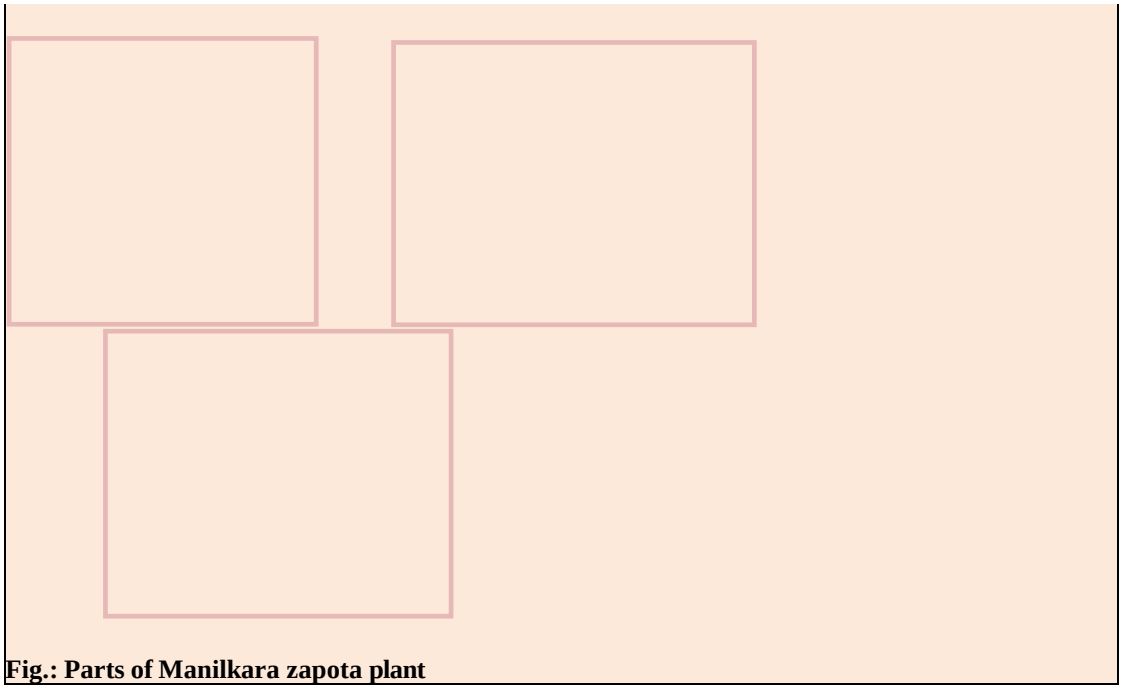

Fig.: Parts of Manilkara zapota plant

Fig. 2.2 Parts of *Manilkara zapota (L.) P. Royen* plants

Traditinal uses:

1) Sapodilla fruit is used as a nutritional food , chicle and also used in many local medicines(Bano M.*et al*.,2017, Karle P.*et al* 2019).

2) A boiled decoction of young fruit of bark is taken to stop diarrhea ,peludism and dysentry.(Bano M.et al.,2017, Karle P.*et al* 2019).

3) Used in tonic Young infusion helps in relieving pulmonary complaints. Melted butter soaked fruit helpful in prevention of biliousness and fevers.(Bano M.*et al.*,2017) Decoction of seed used as a diuretic.(Rupesh P,G.K.D.*et al*.,2015).

4) The content of the unripe fruits helps in resolving stomach problem, also used as a chewing gum (Karle P.*et al 2019*, Bano M.*et al*.,2017).

5) Leaves were also beneficial to treat cough and cold symptoms. Fever wound and ulcers. leaves have good potential for analgesic activity antihyperglycemic and.(BanoM.*et al*.,2017).

Fig: 2.3 Pharmacological activities (Dr. Milind *et al* 2015)

Medicinal uses :

1) While crushed seeds are used to treat stone of bladder and kidney as well as Rheumatism, antipyretic, diuretic also used to treat animal bites. .(Kamaranga P.*et al.2007)*

2) leaf decoction is used to cure fever ,cough ,colds, haemorrage wound and ulcer,sedative and sopoific (Kamaranga P.*et al.2007)*

3) Almost all parts of M. zapota such as leaves .fruit, stem bark and roots posses antioxidant, anticancer, anti diarrheal antimicrobial , antipyretic ,analgesic, anti-inflammatory activity and have good potential for analgesic antihyperglycemic and hypocholesterolemic activities. (Bano M.,*et al* 2017).

Botanical description

Tree: Medium to large tree with a pyramidal to rounded canopy with many branches. In which Young branches are horizontally and dropping. A milky latex knows as "chicle" exudes from all tree parts. (Kamaranga P.*et al.*2007).

Fruit : May be nearly oval, oblate, ellipsoidal in shape as depicted in plate 1.6-9 cm in width hard, gummy and astringent when immature ,coated with a sandy brown scurf smooth -skin until ripe.The flesh is yellowish brown. .(Karle P.*et al 2019)* Friut growth follows a sigmoid pattern. when immature it is hard ,gummy and very astringent. . (Kamaranga P.*et al.*2007).

Leaves: Is an evergreen tree. The leaves of this tree may length from 5-20 cm or 2-5 inches. These are rigid shape and aggregated like bundle at the end of shoots. (Bano M.*et al* 2017) Leaves contain a bitter principle alkaloid and fixed oil. They are medium green, glossy, alternate and spirally clustered at the tip of forked twigs.(Kamaranga P.*et al.*2007).

Flowers: It bears off-white, small, bell-shaped and bisexual .size range from 9.5mm or 3/8 diameter. (Bano M.*et al 2017*) flowers are grrenish ,solitary. (Kamaranga P.*et al.*2007).

***Peel*:** The ripe fruit has a thin,rusty brown,scurfy peel. (Karle P.*et al* 2019).

Seeds: Some fruit are seedless. They are hard and brown or black in colour with one with margine. Seeds contains some phytochemicals like saponin, achras and bitter sapotinine. Seeds also Hydrocyanic acid present

in seed , fixed oil alkaloid so should be removed before eating the fruit. (Bano M.*et al 2017*, Kamaranga P.*et al.*2007).

***Roots* :** Sapodilla is shallow- rooted tree, with more than 80% of the roots located within the top 75 cm of soil, concentrated witin an area half the width of the canopy. (Kamaranga P.*et al.*2007).

Ecological Requirement

Sapodilla is a species of the lowland rainforest. Tree grows well in a wide range of climate condition from wet tropics to dry cool subtropical areas. They prefer a moist hot climate similar to that found at medium to low elevations in tropical areas, such as in coastal regions.

Rainfall: Humid climate with mean annual rainfall of 1250-2500mm

Temperature: range of 10-38ºC is suitable

Altitude: 2800 m in altitude **Soil** : It is well grow in well drained medium, sandy loam and lateritic soil .

Table No: 2.3 Phytoconstituents of chickoo (Parle M.,P. *et al.*,2015 /Karle P.*et al* 2019*)*

Sr. No.	Phytoconstituents	Plant Parts
1	**Triterpenoid:** Erythrodiol	Leaf
2	**Fixed oils:** **Unsaturated oils:** like oleic acid, linolenic acid Lupeol acetate **Saturated oils**: Palmitic acid	Leaf
3	**Hydrocarbons:** n-hexane, n-triacontane, n-octacosane.	Leaf
4	**Sterols:** β-sterol. stigmasterol.	Leaf
5	**Enzyme :** Poly phenol oxidase	Fruit
6	**Alkaloids:** Sapotinine, sapotin	Whole plant
7	**Phenolic compounds:** Quercitrin, myricitrin,(+)- catechin, (-)-epicatechin, (+)- gallocatechin, gallic acid, D-quercitol, saccharose, myricetin-3-α- L-rhamnoside,	Leaf, fruit, seed, peel
8	Ascorbic acid	Leaf , fruit, bark
9	**Minerals:** Iron,copper,zinc,calcium,potassium	Fruit
10	**Carbohydrates:** Lactose,glucose,galactose,fructose,arabinose,sucrose	Leaf , fruit
11	B-caritene	Fruit

12	**Amino acids :** Alanine,arginine,tyrosine ,lysine,aspartic, hydropyroline, isoleucine, leucine,phenylalanine, proline , serine, threonine, valine, methionine, cystine**,**	Fruit, Leaf
13	**Saponine :** Manilkoraside	Stem bark
14	Flavonoid, Tannins, phenolic acids, ellagic acid, flavanols (catechin ,epicatechin), kaempferol, quinic acid, lycopene a carotenoid, Quercitrin, gallocatechin	Peels

Reported pharmacological activities of Manilkara zapota(L.) P. Royen fruit peel

a) Antioxidant activity

The MZFP extract showed its potential as natural antioxidant in *in-vitro* antioxidant assays like 1,1-diphenyl-picrylhydrazyl (DPPH) radical scavenging activity, ß- carotene bleaching activity assays (BCB) and oxygen radical absorbance capacity (ORAC) methods. Antioxidant activity by DPPH scavenging activity had showed a good correlation with phenolic and flavonoid content. While when subjected to *in-vitro* free radical scavenging assays like ABTS+, nitric oxide and lipid peroxi- dation inhibition assays the results indicated that MZFP extract showed highest radical scavenging potential and high antioxidant activity com- pared to that of pulp extracts (Karle P.*et al* 2019)**.**

b) Antimicrobial acivity

The MZFP when subjected to its antibacterial activity using gram negative bacteria (Citrobacter freundii, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis,Salmonella typhimurium and gram positive bacteria (Bacillus megaterium, Bacillus subtilis,Corynebacterium rubrum, Staphylococcus aureus, Staphylococcus epidermidis) and fungi (Candida albicans, Candida glabrata, Candida neoformans, Candida epicola), depicted significant reduction in the zone of inhibition in Gram negative bacteria than other microorganism species. Thus, disclosed a good antimicrobial activity indicating its potency as a promising source of natural antimicrobics (Karle P.*et al* 2019).

Disease profile INFLAMMATION

Definition:
The inflammation term is taken from the Latin word " inflammare"(to burn) (de oliveira (L.A. Abdulkhaleq *et al*., 2018). "Inflammation is biological response of the immune system that can be triggered by a variety of factors including pathogen damaged cells and toxic compound."(Linlin C,Huidan D, H.C, Xung wang *et al.*,2017).

Etiology of Inflammation (Cause) (H.Mohan *et al*.,2010; L.Chen *et al*.,2017).

Infectious agents	Non-infectious agents
Virus, fungi, bacteria, parasite, toxin, other microorganisms.	**Physical:** Burn, heat, cold, radiation, mechanical trauma, physical injury, ionizing radiation.
	Chemical: Organic-Inoraganic poison, Glucose, fatty acid, alcohol, irritants including (fluoride,

	nickel, others trace elements).
	Biological: Damaged cell
	Immunological: Cell mediate and antigen-antibody reaction.
	Psychological: Excitemnt,
	Inert material: Foreign bodies

Types of inflammation:

It occurs in three distinct phases. The first phase which is caused by an elevated vascular permeability resulting in exudation of fluids from the blood into the interstitial space, the second phase occurs by the infiltrations of leukocytes from the blood into the tissue and in third phase granuloma formation and tissue repair (Winter *et al.*, 1962). depending upon the defence capacity of host and duration of response.(H.Mohan *al*.,2010) Inflammation can be either aute or chronic . These are two type of inflammation.

A) Acute inflammation

B) Chronic inflammation

A) Acute inflammation :

In acute inflammation tissue damage due to trauma, microbial invasion or ous compounds can induced acute inflammation.. It start rapidly, becomes severe in a short duration and symptoms may last for a few days for example. celulitis or acute pneumonia.. (Pahawa R.Goyal, A. Bansal.P, *et al*.,2020).

In the inflammatory response, pro-inflammatory mediators such as prostaglandins and leukotrienes play an imperative role (Samuelsson *et al*., 1987). The progression from acute inflammation to chronic inflammation as in many widely occurring human diseases is due to excess of inflammatory mediators (Serhan *et al.*, 2009). The main features of acute inflammation are: .(H.Mohan *et al.*,2010).

1) accumulation of fluid and plasma at the affected site;

2) intravascular activation of platelets; and

3) polymorphonuclear neutrophils as inflammatory cells.

The major cells involve are **neutrophils acute** inflammation characterized by Celsus four cardial signs:-(A.Arora *et al*.2016).

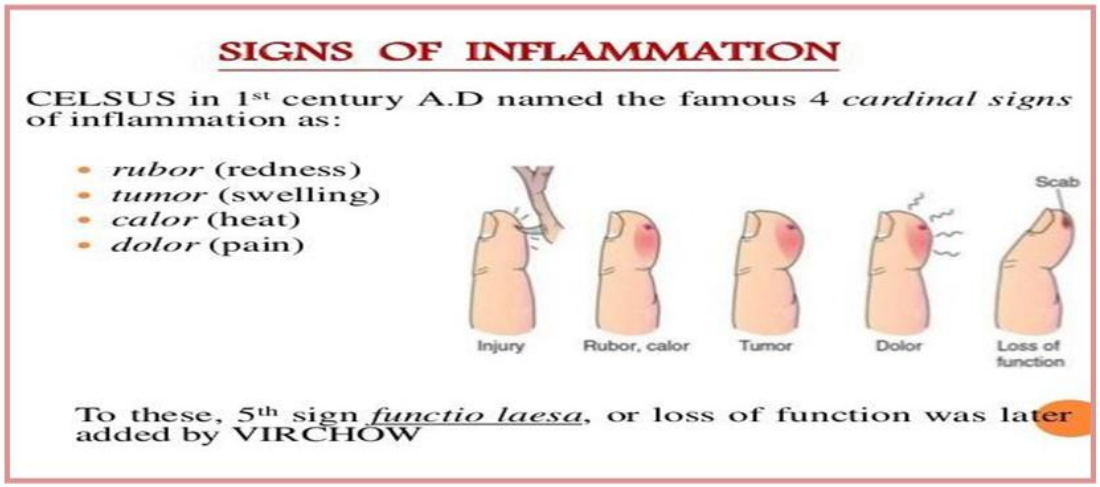

Fig: 2.4 Sign of inflammation.

Acute inflammation has two main components (A. Arora *et al*.2016).

1) Vascular events occure in following sequence:-(A.Arora *et al.*2016).

a) Transient vasoconstriction

It is he earliest step or response to tissue injury (.(H.Mohan *et al.*,2010) transient in nature. It is responsible for **blanching** seen immediately after injury.

b) Vasodilation

It is responsible for **redness (rubor)** and heat (**color)** due to increased blood flow. It first involve arterioles and then result in opening of new capillary beds in the area. There is increased hydrostatic pressure due to increased blood flow.

c) Increased vascular permeability

i) Increased vascular permeability is the hallmark of acute inflammation. This leads to escap of protein rich fluid (exudates) and leukocytes in extravascular space.it is responsible for **swelling (tumor) .**

ii) Intravascular osmotic [ressure reduces due to exudation of protein and osmotic pressure of interstitial fluid is increased.

iii) Most affected vessels are venules.

iv) Mechanism Involved for increased permeability are**:**

- **Formation of endothelial gaps (immeadiate transient response)**

It is the most common mechanism for increased permeability. It occurs due to contraction of endothelial cell cytoskeleton. Important mediator involved are histamine, bradykinine, leukotriene and substance P. Later cytokines (IL-1, TNF, IFN-γ) are also involved. The most commonly affected vessels are venules. The response is rapid,reversible and short lived.

- **Direct endothelial injury (immediate sustained response)**

This response is rapid but long lived. It occurs due to direct injury causing necrosis and detachment of endothelial cells by toxis, infectious or burns. All levels of microcirculation are affected including venules, capillaries and arterioles.

- **Delayed prolonged (sustained) response.**

This response os delayed and long lived. It is caused by miled to moderate thermal injury, X-ray or UV radiation, and bacterial toxins. The mechanism involved is either by direct effects of injuries agents or by cytokine mediated endothelial cell retraction ,caused by cytoskeletal reorganistion. It affects venule and capillaries.

- **Leukocytes mediated endothelial cell injury**

Leukocytes are activated and causes endothelial cell injury. It affects venules (mostly); and pulmonary and glomerular capillaries.

- **Increased transcytosis**

It affects venules. It is caused by formation of vesicular organelles near intracellular junction by histamine and VEGF.

- **Leakage from new blood vessels**

It occurs at the site of angiogenesis as new blood vessels are leaky.

d) Stasis

The loss of fluid from intravascular to extravascular space due to increased permeability) results in concentration of red cells and increased viscosity of blood, leading to slower blood flow and stasis.

2) Cellular Events (Kalpana A.*et al.*,2016)

The cellular phase of inflammation consists of 2 process:

a) Exudation of leukocyte; and

b) Phagocytes.

a) Excudation of leucocytes

The journey of leukocytes from the lumen vessel lumen to interstitial tissue is the most important feature of inflammatory response.

i) In the vessel lumen (changes in the form elements of blood)

In the early stage of inflammation , rate of blood flow is increased due to vasodillation. But subsequently , A stasis or slowing develop, leucocytes principally neutrophills accumulate along the vascular endothelium this is called **migration.** The rows of leucocytes moves (tumble) slowly along the endothelium a process called as **rolling**. Finally coming to at rest, leucocytes adhere firmly to endothelium called **adhesion.** The endothelium can be virtually lined by white cell called as **pavenmenting.**

ii) Dipedesis

It is the process of transmigration of leucocytes across the endothelium. after ferm adhesion , leucocytes migrate through interendothelial junction and assume a position between endothelial cells and the basement membrane. The adhesion molecules include selectin, Intergrins, members of immunoglobulin superfamily ad mucin like glycoproteins.

- **Selectin**

Selectin function in cell to cell interaction i.e., adhesion of leucocytes to endothelium.
E-selectin (CD-62E) is present on endothelium cell and it binds to sialyl-lewis
P-selectin (CD-62P) is present on endotheliu and platelates.binds to sialyl-lewis on leucocytes. **L**-selectin (CD-62L) is present leucocytes. And binds to mucin like glycoproteins GlyCAM-1 on endothelium.

- **Immunoglobulin family**

They are present on endothelium
ICAM-1 (interacellular adhesion molecule-1) binds to **β2**-intergrins of leucocytes. VCAM-1 (vascular cell adhesion molecules-1) binds to **β1**-intergrins of leucocytes.
Platelet endothelial cell adhesion molecules (PECAM or CD-31) is present on both endothelium and leucocytes. It is the major adhesion molecules for diapedisis.

- **Intergrins**

They are present on leucocytes
β1-intergrins (VLA-4) bind to VCAM -1 of endothelium.
β2-intergrins (LFA -1 and MAC-1) bind to ICAM-1 of endothelium.

- **Mucin like glycoprotein**

Present in the extracellular m,atrix and on cell surface Example, Hepaaran sulfate.

iii) Chemotaxix

After the extravasation , leucocytes emigrate into tissue towards the site of injury by chemotaxix. The leucocytes migrate through the pores of filter towords the chemotactic agent. Which may be exogenous or endogenous (C5a,LT-B4,IL-1, TNF,IL-8) C3a- is most powerful chemokine.

b) Phagocytosis

The process of engulfment of solid particulate material by the cells (cell eating). The cell performing this function called as phagocytes. Thye are 2 types

- : Polymorphonuclear neutrophills (PMNs) Microphage
- Monocytes. (macrophage).

B) Chronic inflammation :

Chronic inflammation also reffued to as slow, long term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effect of chronic inflammation vary with the cause of the injury and ability of the body to repaire and overcome the damage. (Pahawa R.Goyal, A. Bansal.P, *et al*.,2020) Cardial sigs of acute inflammation are absent. The main characteristic feature of chronic inflammation are :

1) In chronic inflammation, different cytokines and growth factors are released, resulting recruitment of higher order immflammatory cells such as leukocytes, lymphocytes and fibroblast, granulation tissue formation.

2) The inflammation can lead to permanent tissue damage by these cells (Agarwal *et al*., 2009; Karin *et al.,* 2007).

3) Chronic inflammation can also lead to a number of diseases such as may fever, periodontitis, rheumatoid arthritis, arteriosclerosis, cardiovascular diseases, pulmonary diseases, neurologic diseases and cancer (Nandini *et al.,* 2009; Agarwal *et al.,* 2006).

Types of chronic inflammation

1) Non-specific proliferative chronic inflammation

2) Granulomatous chronic inflammation.

1) Non-specific proliferative chronic inflammation

It is characterized by the presence of non-specific granulation tissue formed by infiltration of mononuclear cells (lymphocytes, macrophage,plasma cells) and proliferation of fibroblass, connective tissue, vessels, and epithelial cells, for examples, an inflammatory polyp-like nasan or cervical polyp and lungs abscess. (Pahawa R.Goyal, A. Bansal.P, *et al*.,2020).

2) Granulomatous inflammation

A specific type of chronic inflammation characterized by the presence of distict nodular lesion or granulomus formed with an aggregation of activated macrophages or its derived cell called epithelioid cells usally surroundedby lymphocytes. The macrophages or epithelioid cells inside the granulomas often coalesce to form langhans or giant cells such as foreign body, Aschoff, Reed-sternberg, and Tumor giant cells. There are two types: (Pahawa R.Goyal, A. Bansal.P, *et al*.,2020).

I) Granuloma formed due to foreign body or T-cell mediated immune response is termed as foreign body granuloma, for example, silicosis. (Pahawa R.Goyal, A. Bansal.P, *et al*.,2020)

II) Granuloma formed due to chronic infection granuloma, for example, tuberculosis and leprosy. (Pahawa R.Goyal, A. Bansal.P, *et al*.,2020).

Available treatment for Inflammation: Non selective Cox inhibitors (Traditional NSAIDs)

1. **Salicylates:** aspirin, Sodium salicylate, diflunisal.
2. **Propionic acid derivatives:** ibuprofen, ketoprofen, naproxen.
3. **Aryl acetic acid derivatives:** diclofenac, ketorolac, etodolac.
4. **Indole derivatives**: indomethacin, sulindac
5. **Alkanones:** Nabumetone.
6. **Oxicams,Enolicam:** piroxicam, tenoxicam, meloxicam.
7. **Anthranilic acid derivatives (fenamates):** mefenamic acid and flufenamic acid, meclofenamic acid.
8. **Pyrazolone derivatives:** phenylbutazone, oxyphenbutazone, azapropazone (apazone) & dipyrone (novalgine).
9. **Aniline derivatives (analgesic ,antipyretic without anti-inflammatory action):** paracetamol, nefopam, metamizol.
10. **Selective COX-2 inhibitor:** Celecoxib ,rofecoxib, lumiracoxib (Ashley et al., 2012; I.Habib *et al.,*

2014; Kalpana A.*et al*., 2016).

Table No: 2.5 Difference between Acute and Chronic Inflammation (H.Mohan *et al*., 2010; Robbin *et al*.,2018).

Feature	Acute inflammation	Chronic inflammation
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non- degradable pathogens, persistent foreign bodies, or auto immune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines,eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days (min, hours)	Up to many months, or years
Cellular infiltrate	Mainly neutrophills	Monocytes /macrophages and lymphocytes
Tissue injury ,fibrosis	Usually mild and self- limited	May be serve and progressive
Local and systemic sign	Prominent	Less

Signs of Inflammation

The five principal signs of inflammation (rubor, tumor, calor, dolor) were described nearly 2,000 years ago by the Roman Aulus Cornelius Celsus, more commonly known as **Celsus** (Robbins *et al.,* 2004) and .(functio laesa) fifth was added in the late 19th century by Rudolf Virchow, knows as the "father of modern pathology ".(Robbin *et al.*,2018).

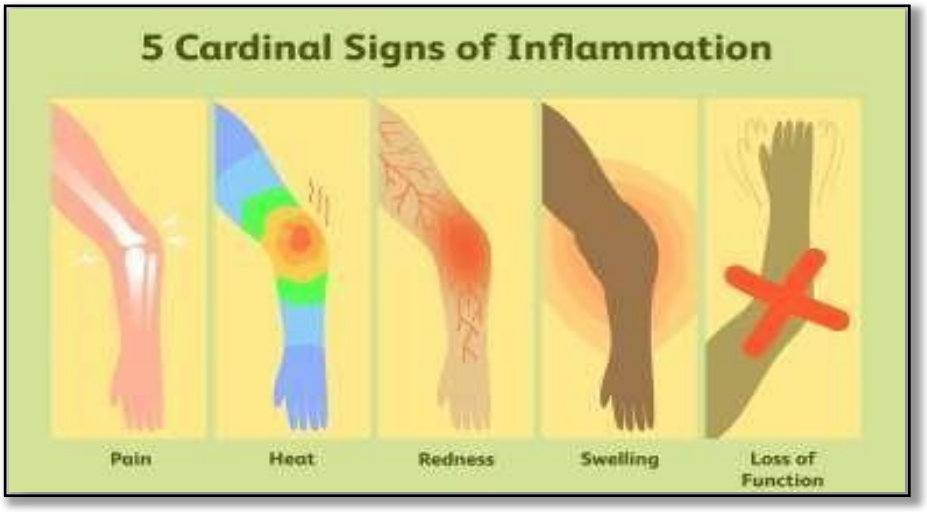

Figure No: 2.5 Five cardinal Signs of Inflammation

1) Redness (rubor)

An acutely inflamed tissue appears red, due to dilatation of small blood vessels within the damaged area which causes heat and redness at the site of inflammation. (erythema) (Robbin *et al.*,2018) Additional number of erythrocytes passing through the dialated vessels which cases redness appeared.(N.punchard *et al*.,2004)

2) Swelling (tumor)

Swelling results from edema, the accumulation of fluid in the extravascular space as part of the inflammatory fluid exudate, and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the area (Robbins *et al.,* 2004). Increases passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area,and prolonged inflammatory response deposition of connective tissue.(N.punchard *et al*.,2004)

3) 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 (Tracy et al., 2019).or the sensation of heat is caused by the increased movement of blood through dialated vessels into the environmentally cooled extremities.(.(N.punchard *et al*.,2004)

4) 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 (Rather *et al*., 1971; (N.punchard *et al*.,2004)

5) Loss of function (functio laesa)

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 (Tracy *et al.,* 2019). Loss of mobility in joint ,due to th oedema and pain, or to the replacement of functional cells with scar tissue (N.punchard *et al*.,2004)

Role of Arachidonic acid in Inflammation

The potent mediators of inflammation are derivatives of arachidonic acid (AA) a 20- carbon poly unsaturated fatty acid produced from membrane phospholipids. Arachidonic acid, the major poly unsaturated fatty acid present in mammalian systems is the precursor for prostaglandin synthesis by cyclooxygenase pathway. Under normal conditions the concentration of free arachidonic acid AA within the cells is low. Most of it is stored as part of phospholipids in the membranes of the cells (Brash *et al.,* 2001). In most cells, arachidonic acid released at the endoplasmic reticulum and nuclear membrane, predominantly via the translocation of type IV cytosolic phospholipase A2. arachidonic acid released from the membrane is rapidly metabolized in several enzymatic and non-enzymatic pathways to yield an important family of oxygenated products, collectively termed eicosanoids (Simmons *et al*., 2004). The arachidonic acid metabolism generally occurs via one of four major avenues:

1) The cyclooxygenase (COX) pathway, involved in the formation of prostaglandins (PGs), thromboxanes (Txs), and prostacyclin.
2) The lipoxygenase (LOX) pathway, which produces leukotrienes (LTs) and lipoxins.
3) The cytochrome P450 mono oxygenase pathway, which produces epoxyeicosatrienoic and hydroxyeicosatetraenoic acids.
4) Non-enzymatic lipid peroxidation which produces isoprostanes (Howard *et al*., 2006).

CHEMICAL MEDIATORS OF INFLAMMATION

The vascular and cellular events of both acute and chronic inflammation are mediated by chemical mediator,called mediators of inflammation. These mediator originate: (A.Arora *et al.,* 2016).

1) From cell: (Cellular mediators): They may be :-

Performed: Histamine ,serotonin, lysosomal enzymes. Newly synthesized: Prostaglandin ,leukotrienes, Thromoxane, platelete activating factors, nitric oxide, cytokines(IL-1, TNF- α). (A.Arora *et al.,* 2016).

2) From plasma: (Complement protein, clotting factor ,kinin system (including bradykinin, (A.Arora *et al.,* 2016)

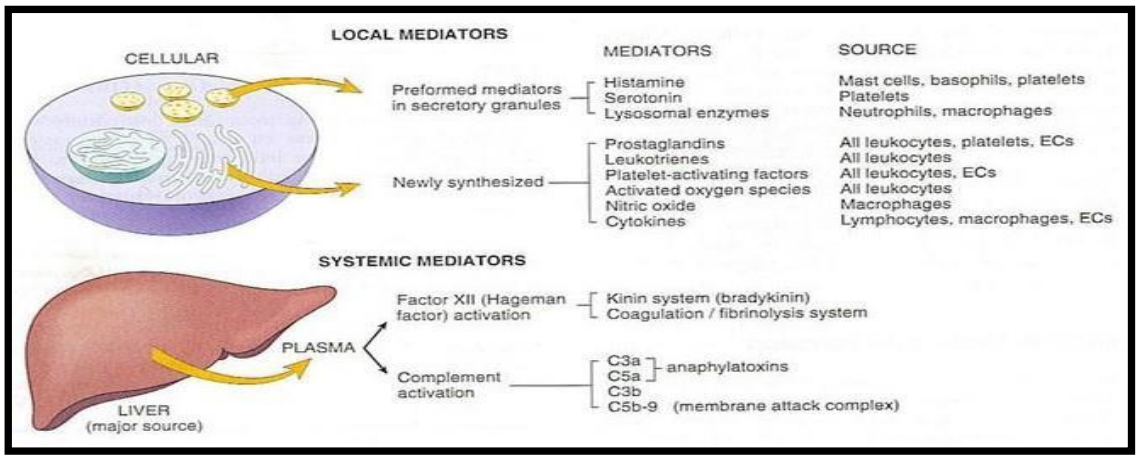

Fig No: 2.6 Chemical Mediators of inflammation

A) CELL DERIVED MEDIATORS

1) Vasoactive amines

Two major vasoactive amines, so named beacused they have important action on blood vesselsare histame and serotonin. They are first mediator to be relased during inflammation.(Robbins *et al.*,2018).

- **Histamine: (β-Imidazolyethylamine)**

Richest source of Histamine is synthesized in a Golgi apparatus of must cell and basophills to maintain acute-phase response during inflammation events (L.A.Abdilkhaleq *et al.*,2018) by decarboxylation of the amino acid histidine and is then stored in secreatory granules in complex with heparin, protein,or both.(M.White *et al.*,1999). Normally present in connective tissue and blood vessels.(Robbins *et al.*,2018). The main action of histamine are vasodialation, increased vascular (venular) permeability, smooth muscle contraction , itching and pain. Stimulation of must cell and basophills also released products of arachidonic acid metabolism including the release of slow-reacting substance anaphylaxis it consist of various leukotriens.(LTC4,LTD4 and LTE4). (H.Mohan *et al*.,2010) It exerts its its effects on a variety of cell types (endocrine and exocrine),,blood cells, and cell of immune systems. .(M.White *et al*.,1999).

- **Serotonine (5HT or 5-hydroxytryptamine)**

It is presend in platelets and tissue like chromaffin cells of GIT, spleen, nervous tissue, mast cells .Biological effects of serotonin are same as histamine but it is less potent mediator of increased vascular permeability and vasodilation than histamine (A.Arora *et al.*,2016; H.Mohan *et al*.,2010). It is produced via decarboxylation of tryptophan, and it is stored in granul.Four serotonin receptors,namely 5-HTI,5-HT2, 5-HT3,and 5- HT4,were documented to mediate its biological functions. (L.A.Abdilkhaleq *et al.*,2018).

- **Neuropeptide**

Another class of vasoactive amine is tachykinin neuropeptide, such as substance P, neurokinin A,vasoactive intestinal polupeptide (VIP) and somatostatin. These are small peptides are produced in the central and peripheral nervous systems.(H.Mohan *et al*., 2010) Neuropeptides constitute one of the largest families of extracellular messengers, having a long phylogenetic history. They can act as nrurotransmitter ,hormones, and paracrine factors.(M.schaffer *et al*., 2020).The major proinflammatory *actions* of these neuropeptides is as follows:.(H.Mohan *et al*.,2010).

a) Increased vascular permeability.
b) Transmission of pain stimuli.
c) Mast cell degranulation

2) Platelet-activating factor

PAF it is derived from cell membrane phospholipids by the action of PLA2. PAF, synthesized by mast cells, platelets, neutrophills, endothelium macrophages, and eosinophils, inducees platelets aggregation and stimulates platelets to release vasoactive amine and synthesize thromboxane. PAF also increase vascular permeability, cause neutrophills , platelets aggregate and degranulates. (H.Mohan *et al*.,2010) vasodilation in low

concentration. and vasoconstriction, bronchoconstriction, adhesion of leucocytes to endothelium.(Scott H.Edwards *et a.,l* 2014).

Platelet activating factor (PAF) is an ether-linked phospholipid, designated as such because of its discovery as a basophil-derived mediator of rabbit platelet activation. The synthesis of PAF occurs as a 2-step pathway in which phospholipase A2 hydrolyzes a 1-O- alkyl-2-acyl-glyceryl- 3-phosphorylcholine to produce 1-O-alkyl-2-acylglyceryl- 3- phosphocholine (lyso-PAF), which is then converted by an acetyl transferase enzyme to PAF. The biological activities of PAF include platelet activation, activation of neutrophils, and smooth muscle contraction. Because PAF is rapidly inactivated *in vivo*, it is likely that it triggers a chain of inflammatory events. PAF stimulates the accumulation of eosinophils to endothelial surfaces, which may be the first step in the recruitment of eosinophils into tissues. Eosinophils also are a source of PAF and can attract additional eosinophils, which can potentiate the inflammatory reaction. PAF stimulates eosinophils to release basic proteins, leading to epithelial cell damage, and causes an increased expression of low-affinity IgE receptors on eosinophils and monocytes (White *et al.,* 1999).

3) Free radical) Nitric oxide (NO)

It is also called Endothelium-derived-relaxing factor(EDRF) It is widely known that nitric oxide (NO), synthesized from amino acid L- arginine by Action of enzyme nitric oxide synthase (NOS) which is cytosolic.(A.Arora *et al.,* 2016)

NO synthase

Arginin Citruline + NO ⟶

Cytosolic

It is most important physiological source of endothelial cells. .(A.Arora *et al.,* 2016)It is important cell signaling messenger is involved in diverse physiological and pathological proces (Scott H.Edwards *et a.,l* 2014). An excess in NO production is largely thought of as causing a variety of inflammatory diseases, such as sepsis, psoriasis, arthritis, multiple sclerosis, and systemic lupus erythromatosus Vasodilation (Abdulkhaleq *et al.,* 2018). Play roll in maintaining resting vascular tone, vasodilation, antiaggregation of platelets. .(Scott H.Edwards *et al.,* 2014) vascula relaxation factor produced by endothelial cells. Now it is known that NO is formed by activate macrophages during the oxidation of arginine inflammation (H.Mohan *et al.,* 2010).

- Vasodilation
- Anti-platelet activating agent
- Possibly microbial action.

4) Cytokines

Cytokines are small soluble secreate protein (polypeptide) substance it produced by lymphocytes, and also activated monocytes. Other names of cytokines are (Lymphokines, monokines, chemokines) .cytokines made by lymphocytes. cytokines can also act synergistically and antagonistically .(Jun-Ming Zhang *et al*.,2007) They agents may action "self"cells producing them or on other cells. They mainly involved in immune response, but some cytokines may also take part in inflammation.(H.Mohan *et al.,*2010) They are made by many cells population, but the predominant producers are helper T-cells and macrophages. Cytokine may produced in and by peripheral nerve tissue during physiological and pathological process .(Jun Ming Zhang *et al.,*2007)

a) Proinflammatory cytokines :

- These are involved in amplification of inflammation. Major proinflammatory cytokines are IL- 1, TNF-α and IL-6. Other proinflammatory cytokines are IL-2, IL-4 , IL- 6, IL-8, IL-11, IL-12, IL-15, IL-21, IL-23,IFN-γ and GM-CSF. .(A.Arora *et al.,* 2016)
- IL-1 is most important cytokines responsible for systemic effects of inflammation.(.(A.Arora *et al.,* 2016)
- Interleukin(IL-1) and Tomour (TNF) –α found by activated macrophages. .(H.Mohan *et al.,*2010).

- Tomour (TNF-β and Interleukin (IFN)-γ are produced by activated T-cells. .(H.Mohan *et al.*,2010).
- Chemokines include interleukin 8 release (from macrophages) and platelets factor -4 from platelets. Boths are potents chemoattrctant for inflammatory cells. .(H.Mohan *et al.*,2010).

b) Antiinflammatory cytokines:

- These involved in resolution of inflammation .Example are IL-4, IL-10, IL-13 and transforming growth factor β (TGF-β). IL-4 has mainly anti-inflammatory property with some proinflammatory action. TGF-β is the most important fibrogenic factor. .(A.Arora *et al.*, 2016). Action of various cytokines are as follows:

i) **IL-1 and TNFα, TNF-β** induce endothelial effects in the form of increase leucocytes adherence,thromagenecity,elaboration of other cytokines, fibroblastic proliferation and acute phase reaction. .(H.Mohan *et al.*,2010)

ii) **IFN-γ** cause activation of macrophage ,neutrophills and associated with synthesis of nitric acid synthase. .(H. Mohan *et al.*,2010)

iii) **Chemokines** –It is family of chemoattractant for inflammatory cell are follows; .(H.Mohan *et al.*,2010).**IL**-8 chemotactic for neutrophill Platelets factor -4 chemotactic for neutrophills,monocytes and eosinophils. MCP-1 chemotactic for monocytes an Eotaxin chemotactic for eosinophills.

5) Tumor necrosis factor (TNF-α):

Over production of TNF-α can lead to autoimmunity, malignancy or inflammatory and immunopathological disease. Thalidomide (alpha-N-pthalimidoglutarimide) is an immuno modulator and anti-inflammatory drug. It is clinically useful in a number of conditions through its ability to inhibit selectively TNF-α synthesis. It is the drug of choice in the treatment of erythema nodosum leprosum an acute inflammatory complication often seen in patients with lepromatous leprosy, rheumatoid arthritis and HIV (Barbara *et al*., 1996; Tracey *et al*., 1995).

B) PLASMA- DERIVED MEDIATORS

6) Kinin

Kinin system includes prekallikreins ,kallikreins ,and kinin. These system activated by factor of XII (Hagemans factor) which convert prekallikreins into kallikreins. Kallikreins, then converts kininogen into bradykinin. (A.Arora *et al.*, 2016) (Kinin it is the group of small potent vasoactive pepetides which is formed in blood and biological fluids and tisues during inflammation. They are derived from plasma proteins such as a2 globulin called as kininogen. (high and low molecular weight) by the action of proteolytic enzymes called as kallikreins. The kallikreins cleaves a plasma glycoproteins precursor, high molecular weight kininogen, to produce bradykinin. They increases vascular permeability and causes smooth muscle contraction, dilation of blood vessels, and pain injected into the skin.(Robbins *et al*.,2018) Three distinct kinin have been identified in human Bradykinin, kallidin (Lys-bradykinine), and met-Lys bradykinin. (M.White *et al.*,1999).

When physiologic or pathophysiological stimulus activates the proteolytic enzmes (kallikreins) the nanopeptide bradykinin is formed in the blood from high molecular Weight globulin(plasma pathway). Similarly, the decapeptide kallidin (lysyl bradykinin) is released in tissue by activation of the kallikreins on low molecular weight kininogen (tissue pathway).(Michel Schaffer *et al*.,2020). Kinin are naturally accuring agent involved in inflammatory reactions. Examples, vasodialtion, increase vascular permeability, an mobilization of blood and tissue cells. There is evidence that they kinin effects are, mediated by tachykinins released from Sensory nerve ending. Blockade of the kinin B2 receptors with a selective antagonist had an inhibitory effects on plasma extravasation in the trachea and nasal mucosa caused by antigen challenge in guinea pig.(Michel Schaffer *et al*.,2020).

7) Bradykinin

Bradykinin is nanoparticle produced from plasma Kiin-Kallikreins systems. Two or are distinct receptor are present for bradykinin which have been titled B1 and B2. Similar to histamines and serotonin, it can increases the prostaglandin synthesis and produced pain locally.(L.A.Abdulkhaleq.*et al.*, 2018). The kallikreins cleaves a plasma glycoproteins precursor, high molecular weightkininogen, to produce bradykinin. They increases vascular permeabilityin in Acute inflammation) and causes smooth muscle contraction, dilation of blood vessels, and pain injected into the skin. It is also involved in the mechanism of pain. (Robbins *et al*.,2018) .

8) Eicosanoids

Eicosanoids are amphipathic bioactive singnalling (Roger G.B. *et al*.,2018) more potent lipid molecules (from the greek eicosa=twenty; for twenty carbon fatty acid derivatives C.D. Funk *et al*.,2001) derived from arachidonic acids. They are involved in numerous homrostatic biological function and inflammation.(C.D. Funk *et* al.,2001).(AA), During inflammation ,various biologically lipid mediators are active and produced from membrane phospholipid. (A.A. Kalpana Arora *et al*., 2016).It is major constituent of membrane phospholipid.

- **Arachidonic acid metabolites**

Arachidonic acid or eicosatetraenoic acid it is 20-carbon polyunsaturated fatty acid derived from dietry source or by conversion from the essential fatty acid (linoleic acid)It is lipid mediator p It is does not occur free in cell but is normally esterified in membrane phospholipid. When Phospholipase A2 is activated by mechanical trauma or by specific cytokine,growth factor other stmuli. they stimulate vascular and cellular reaction in acute inflammation. C.D.Funk *et al*.,2001) it released arachidonic acid from membrane phospholipids. From arachidonic acids, mediators of inflammation are synthesized by following Pathways(A.A. Kalpana Arora *et al*., 2016).The metabolism of Arachodinc acid takes place by oxygenation through four separate patways: (M.Donowitz *et al*.,1984).

- **Metabolite via Cyclo-oxygenase pathway (COX-1 ,COX-2)**

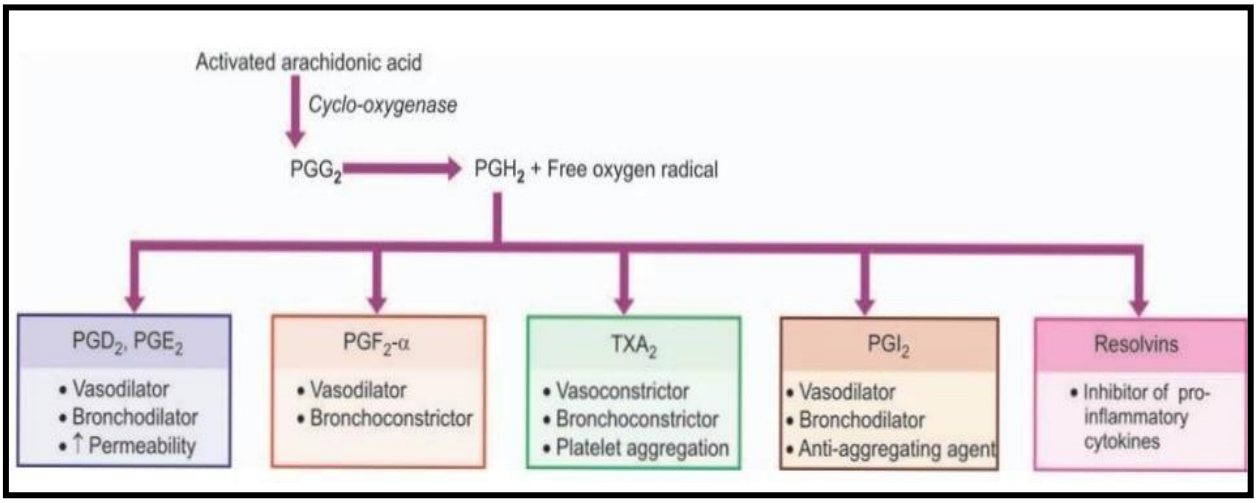

Fig No: 2.7 (COX) pathway

COX is an enzyme involved in synthesis of proteinoids including potent proinflammatory prostaglandin and Thromboxane A2.Two types of cyclooxygenase pathways (COX-1constitutive enzyme not inducible and COX-2 inducible enzyme) also know as PGH synthase. Convert arachidonic acid to PGG2 and then to PGH2. Are called as cyclic endoperoxidase. (Kalpana .Arora *et al*.,2016). Cyclooxygenase also known as Prostaglandin endoperoxide H2 synthase (PGHS) and exists in two isoforms; PGHS-1 (COX-1) and PGHS-2 (COX-2) reacting on free AA to prostanoids. (V.S.Hanna *et al*.,2018). COX-1 and COX-2 enzymes are heme proteins, homodimers that are widely distributed. These enzymes are located in the lumenal portion of the endoplasmic reticulum membrane and the nuclear envelope. (Abdulkhaleq *et al*., 2018). COX-2 mostly in the nucleus ,kidney, brain stomach, forebrain, uterine epithelium, (Abdulkhaleq *et al*., 2018).

Therefore, it appears that COX-1 and COX-2 are two distinct prostanoid biosynthetic systems with separate biological functions for their products. COX-1 mediated TXA2 production regulate platelet aggregation While COX-2 mediate PGI2 release inhibit platelets aggregation and promote vasodilation. TXA2 production regulate platelet aggregation COX-1 is exp.ressed constitutively in most mammalian tissues and plays a role in the production of PGs that control normal physiological processes such as regulation of gastric response and haemostatis and maintain of kidney function.(Kalpana Arora *et al*.,2017). Therefore, it is kept responsible for the housekeeping prostaglandins synthesis. In contrast, COX-2 is an inducible enzyme responsible for the production of pro-inflammatory PGs causing inflammation and fever, pain (F .Seta *et al.,* 2012; I.Habib *et al.,* 2014).

1) Prostaglandin

PGs are small lipid molecules that regulate numerous process in the body .including kidney function , platelets aggregation neurotransmitter releases and modulation of immune function.(H.G.Sarah *et al*.,2002) Prostaglandin and related compounds are called as ***autocoids*** because these substance are mainly auto and paracrine agents. It is the group of arachidonic acid –derived molecule that mediate allergic reaction are prostaglandins.

The the most abundant cyclooxigenase product generated by the immunological activation of human lungs mast cell is PGD2.The biological action of PGs generated during mast cell –dependent reaction in tissue include modulation of smooth muscle contractility, vascular permeability, sensation of pruritus, pain and platelet aggregation and degranulation.
.(M. White *et al*., 1999;.C.D Funk *et al*., 2001) PGs are generated begins with the liberation of AA from membrane phospholipid by phospholipase A_2 in response to inflammatory stimuli. By the action of the action of COX an enzymes associated with endoplasmic reticulum of mast cells.(H.G.Sarah *et al*.,2002) .Prostaglandins (PGs) are carboxylic acids containing 20-carbon atoms. They like hormones and act as chemical messengers. They work within the cells where they are synthesized. (Baraniuk *et al.*, 1990).. Prostaglandins activate the inflammatory response, production of pain, and fever. (J.R. Vane *et al.*,1998) Once formed, PGH_2 is acted upon by a series of enzymes that produce biologically active 3 metabolites PG, TX and PGI with different isomerise such as PGD2,PGE2,PGF2-α. called prostanoids.(C.D.funk *et al.*,2001).

PGD_2 : PGD_2 is major prostaglandin made by mast cell along with PGE_2 . PGD2 also is chemoattractant for neutrophills. Act on blood vessels and bronchodilation an inhibit inflammatory cell function which causes increases vascular permeability, vasodialation and resultant edema, Allergic inflammation, chemoattractant fo neutrophills (Robbins *et al.*,2018; F.seta *et al*.,2012 ;(V.S.Hanna *et al*.,2018).

PGE_2 : Produced by fibroblast, kidney, lungs, macrophages and exert the action by binding to one of its 4 subtype of receptors.(EP_1,EP_2 EP_3, EP_4)(H.G.Sarah *et al*.,2002) Act on blood vessels and bronchodilation and inhibit inflammatory cell function which causes increases vascular permeability, vasodialation and resultant edema, pain sensation (Robbins *et al.*, 2018(V.S.Hanna *et* al.,2018).

PGF2-α: induces vasodilation and bronchoconstriction.

PGI2 (prostacyclin) : Produced by vascular endothelial cells . potent Inhibitor of platelets aggregation to serves to prevent thrombus for on normal endothelial cells. and promote vasodilation. It has role in allergic imflammation thrombus formation on normal endothelial cells. and promote vasodilation. (Nagai *et al*., 2010; V.S.Hanna *et al*.,2018).

2) Thromboxane A_2 (TXA_2)

Thromboxane is an arachidonic acid metabolite with a chemical half life of about 30 sec TXA2 produced during the catalysis of AA by COX pathway from membrane Phospholipid. Thromboxane is first found in platelets as a microbial enzyme, N.Nakahata *et al*.,2008) Thromoxane is produced from PGH_2 through the action of A_2 Synthase also found in lungs, fibroblast, spleen, kidney,colon, stomach in (pig), brain leucocytes, macrophages, endothelial cells.(Roger G.B. *et al.*,2018) The weight of TX is 60 KDa and it is preffered substrate is AA.(N.Nakahata *et al*.,2008). Platelets contain the enzyme TX synthetase and hence metabolite TXA_2, formed is active in platelets aggregation, best Platelets represent the best known cell type to produce TXA_2 in response to various stimuli. However, many other cells and tissues are also able to synthesize is best role as a and bronchoconstrictior, active vasoconstriction. Smooth muscle contraction (Abdulkhaleq *et al.*, 2018; C.D.funk *et al*., 2001).

- **Metabolite via Lipoxygenase pathaway (LOX-5-HETE, Leukotriene, Lipoxin) There are 3types of LOX pathway**

a) 5-Lipoxygenase:- It acts in the presence of FLAP a membrane associate transfer proein (5- LOX activity protein and generates Leukotriences (LTC_4,LTD_4, LTE_4).

b) 12-Lipoxygenase:- converts AA to 12 HETE and further Hepoxillins.

c) 15-Lipoxygenase :- It is involved in the generation of Lipoxin i.e., Lipoxin (LXA_4) and (LXB_4). .Lipoxin.(Kalpana . Aroa *et al.*,2016).

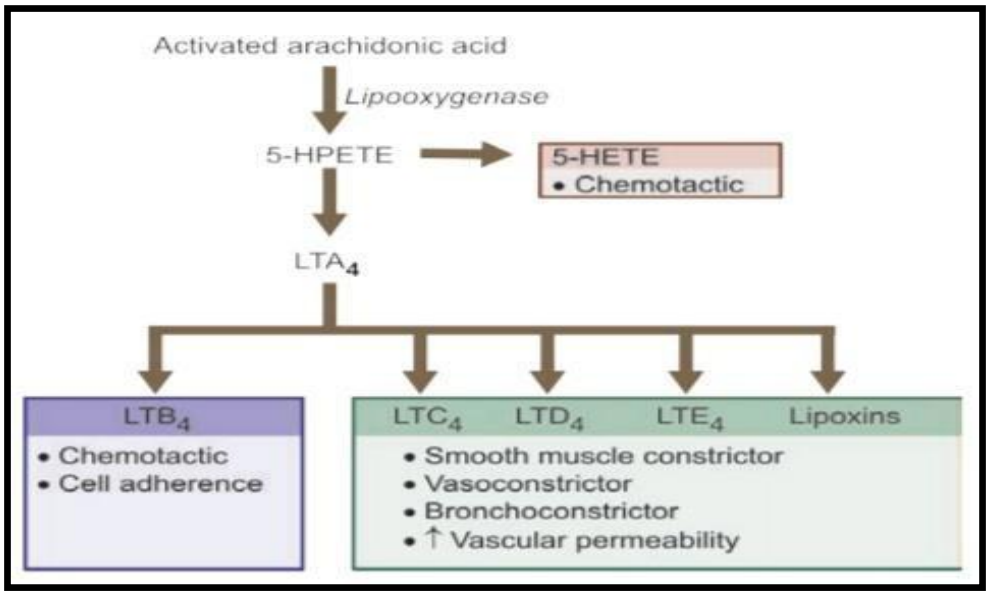

Fig No: 2.8 (LOX) pathway

3) Leukotriene

This enzyme has been found in eosinophils, neutrophils, monocytes, macrophages, mast cells, basophils, and B lymphocytes (Schindler *et al.,* 1998). Leukotrienes (LT) are eicosanoid lipid mediators, which may be responsible for the effects of an inflammatory response (Bailey *et al*., 1985). The name "Leukotriene" was introduced by the Swedish biochemist B. Samuelson, from the words leukocyte and three conjugated double bonds. Leukotriene are so named as they were first isolated from leucocytes. The leukotrienes are formed by the transformation of Arachidonic acid into an unstable 5-HETE intermediate.by lipoxygenase enzymes.

Leukotriene A4 (LTA4) ,which is converted to LTB4 by hydration. It gets converted to LTC4 by the addition of glutathione. This LTC4 is metabolized to LTD4 and LTE4 by the successive elimination of gamma glutamyl residue and glycine. LTB4 is an important mediator of inflammation. LTD4 and LTE4. Makeup biological mixture of sulphidopeptide previously known as slow-reacting substance of anaphylaxis. (John A.Salmon *et al.*,2020; M.White *et* al.,1999). It is a potent chemotaxin for neutrophils and increases leukocyte adhesion to the blood vessel walls (Samuelson *et al.,* 1987). are synthesized and released from the mast cells and basophils following allergen challenge (Naclerio *et al*., 1991; Borgeat *et al.,* 1979 ; B.Samuelsson *et al.*,1987).

Leukotrience	**Biological action**
LTB4 Leukotriene	Leukocyte adhesion, chemotaxix, degranulation
LTC4, LTD4, LTE4	bronchoconstriction, increase vascular permeability, Vasoconstriction, Astama
Lipoxin LXA4, LXB4	Vasodilation,Inhibit neutrophill ,Chemotaxic

- **Lipoxin**

Lipoxin generated from AA by the Lipoxygenease pathway , but unlike prostaglandin and leukotriene, the lipoxin suppress inflammation by inhibiting the recruitment of leukocytes. They inhibit neutrophills chemotaxis and adhesion to endothelium. (,B.Samuelsson *et al.,* 1987).

Outcome of acute inflammation

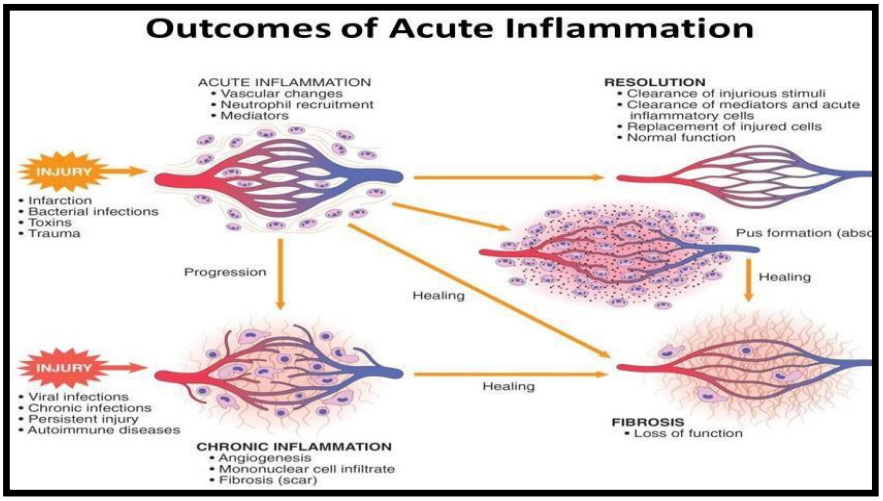

Fig No. 2.9.Outcome of acute inflammation

1. Complet resolution

In a perfect world, all inflammatory reaction, after they have succeeded in eliminating the offending agent, should end with restoration of the site of acute inflammation to normal. This is called as resolution and it is usual outcome when the injury is limited or shot- lived or when there has been little tissue destruction and the damaged parenchyamal cells can regenerate. Resolution involves removal of cellular debris and microbes by macrophages, and resorption of edema fluid by lymphatics.

2. Healing by connective tissue replacement (scarring or fibrosis)

This occurs after substantial tissue destruction, when the inflammatory injury involves tissue that are incapable of regeneration, or when there is abundant fibrin exudation in tissue or in serious cavities (pleura, peritoneum) that cannot be adequately cleared. In all situation, connective tissue grows into the area of damage or exudates, converting into a mass of fibrous tissue.

3. Progression of the response to chronic inflammation

Acute to chronic transition occurs when the acute inflammatory response cannot be resolved, as a result of either the persistence of the injurieous agent or some interferences with the normal process of healing (Robbin *et al*.,2018).

ANALGESIC

Definition:

"Aalgesics are the painkiller substances, which act by the absences of pain without losing consciousness**".** The word analgesic derived from greek *an* **("without ")** and algos **("pain").** Pain can be defined as **"**somatic sensation of acute discomfort, a symptoms
of some physical hurt or disorder , or even emotional distress. Pain is crucial aspect of body defence mechanism and it is part of a rapid warning relay instruction the motor neurons of the central nervous systems to minimize physiscal harm.**"**(M. Kumar *et al*.,2010).

Type of pain

1) Acute pain : Acute pain "it is the body's warning of present damage to tissue or diseases. It is fast and sharp followed by aching pain. It is short term pain or pain with easily identifiable cause.

2) Chronic pain : Chronic pain last much longer than pain normally would with a particular injury. Chronic pain can be constant and intermittent and is generally harder to treat than acute pain. Pain can also be grouped by its source and related pain detecting neurons such as cutaneous pain, somatic pain, visceral pain and neuropathic pain(A. Shete *et al*.,2010).

Causes of pain

a) Cause by stimulation of pain receptor which are free nerve ending.

b) pain receptor located outside the spinal column in the dorsal root ganglion and are named based upon

their appearance at their sensory ends. Sensory end look like bransches of small bushes.

c) Perception of pain is when these receptor are stimulated and they transmit signal to the central nervous system via sensory neuron in the spinal cord (Z. Akbar *et al*.,2010).

Meachanism action of analgesic

Analgesic system is mediated by 3 components:

a) The periaquaductal grey matter (in the midbrain)

b) The nucleus raphe magnus (in the medulla)

c) Pain inhibitory neuron within the dorsal horns of the spinal cord, which act to inhibit pain-transmitting neuron also located in the spinal dorsal horn (M.Kumar *et al.*, 2010).

The management and treatment of pain is probably one of the most common and yet difficult aspects of medicinal practice. Analgesic therapy is domain by two major classes of analgesic drugs; viz. opioids and non-steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbance, renal damage (with NSAIDs drugs), etc. (Dahl *et al.*, 2000; Bures *et al.*, 2002).

Sources of analgesic drugs:

1) Natural source :

There are various medicinal plants are available in nature which shows analgesic activity ,these are follows (M. Kumar *et al.*,2010).

a) Opoid analgesic **:** opoids are any medication which bind to opoid receptors in central nervous systems which shows analgesic activity. Derived from opium from juice of opium poppy, *papaver somniferum*. Act as strong analgesic , for relief of chronic pain. **Eg**. Codein morphine (M. Kumar *et al*., 2010 ; A. shete *et al*.,2010).

b) Other medicinal plants **: Eg.** Aloevera Barbedensis , Punica granatum (flower), Manilkara zapota (leaves, root).

2) Synthetic source :

Paracetamol, diclofenac sodium, Ibuprofen, COX-2 inhibitors, NSAIDs (Z .Akbar *et al.*, 2010; M. Kumar *et al.*,2010).

OXIDATION

The utilization of oxygen to produce energy through the metabolism of food nutrients acts as a prerequisite for the survival of all living beings. While oxygen is one of the most essential components for living. It is also highly reactive atom that is capable of becoming part of potentially damaging molecules such as hydroperoxyl radicals, superoxide anions, singlet oxygen, hydrogen peroxide, organic peroxide, nitric oxide , triplet oxygen. Oxygn uptake while breathing cause free radical production and in addition to the environmental factors such as pollutants, smoke and certain chemicals also contributes to their formation.In turn radicals or death to the cell. This process also takes places in food matrix that contain higher amount of lipid and affects it stability. The components that have the antioxidant property were intentionally added in lipid foods to terminate the chain reaction by removing free radicals intermediate and inhibit other oxidation reaction.(P.Anbudhasan *et al*.,2014).

Fig No. 3 Sources of free radicals.(Oxidation)

FREE RADICAL

"Free radical are molecules or chemical compound which contain an unpaired electron spinning on peripheral layer around the nucleous. Due to the presence of a free Electron" (S.V.Chanda *et al*.,2010) these molecules are highly reactive and unstable. They are important intermeiated in natural process involved in cytotoxicity, control of vascular tone, and neurotransmission.(A.D.Sarma *et al*.,2010). Or

"Every second of our life, our cells are bombarded by particles called free radicals". Normally they protect us from virus, bacteria and other foreign substance. When our antioxidant defences are adequate, damage caused by those free radical is repaired without many consequences. However when excessive amount of free radical generates it can damage protein lipid enzymes and DNA that can alter downstream cell signaling and a cause variety of diseases (Khanna H.D. *et al.*,2014).

Since humans or human ancestors first evolved, a destructive class of chemical agents has assailed the human body. They are called "free radicals", though they are also termed "reactive oxygen species" and abbreviated to "ROS". The free radicals come from oxygen and highly oxygenated molecules (Sulekha *et al.*, 2009).Electrons normally exist in pairs in specific orbitals in atoms or molecules. Free radicals, which contain only a single electron in such any orbital, are usually unstable toward losing or picking up an extra electron, so that all electrons in the atom or molecule will be paired. Free radicals can be positively charged, negatively charged, or neutral. The presence of an unpaired electron in an atom or molecule provides great reactivity, thus shortening its half-life (Rao *et al.*, 2006; Freeman *et al.*, 2003).

Formation of free radicals

Normally, bonds don't split to leave a molecule with an odd, unpaired electron. But when weak bonds split, free radicals are formed. Free radicals are vey unstable and react quickly with other compounds trying to capture the needed electron to gain stability. When the "attacked" molecules loses its electron, it becomes a free radical itself, beginning a chain reaction. All this happen in nanoseconds. Once the process is started, it can cascade, finally resulting in the damage of a living cells. Some free radicals may aries normally during metabolism and by immune system cells to neutralize viruses and bacteria. Normally, body can handle free radical, but if antioxidants are unavailable ,or if the free radical production becomes excessive, damage can occurs (Adheri D.Sarma *et al.*, 2010).

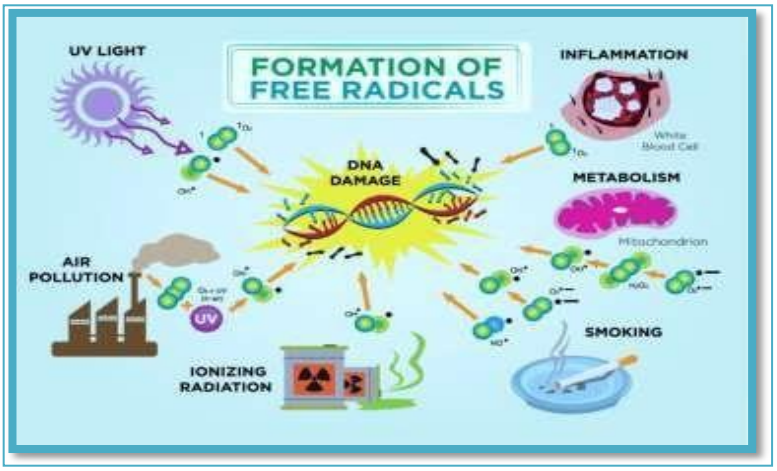

Fig No 3.1 Formation of free radical

Free radicals formed by 3 ways

Mechanism for the formation of free radicals (P.Anbudhasan *et al*.,2014)

1) Homolytic cleavage of covalaent bond of normal molecule, with each fragment retaining one of paired electron .

X : Y X*+Y* ⟶

2) Loss of single electron from normal molecules.

X:Y **$X^{-} + Y^{\cdot}$** ⟶

3) Addition of single electron to normal molecules.

X + e **$X^{\cdot}$** ⟶

Steps involving free radicals generations

1) Initiation: Reaction are those ,which result in increase in number of free radicals. They involved in formation of free radicals from stable species or the may involved reaction of free radicles with stable species to form more free radicles.

2) Propagation: Reaction involve free radicals in which the total number of free radicals remains the same.

3) Termination: Those reaction resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, For example: 2Cl Cl2 (Abheri D.Sarma *et al*.,2010).

Free radical-targets

Free radicals attact three main cellular components

a) Lipid

Membrane lipid are the major target of mitochondrial ROS. The OH radical interacts with unsaturated bonds in a membrane lipid and start the process of lipid perioxidation .The end product of this reaction is 4-hydroxynonenal, a compound that affect the activity of various membrane proteins. It is the major inducer of oxidative stress and has been associated with a variety of pathophysiological states.(S.Biswas *et al*.,2017). Peroxidation of lipid in cell membrane can damage cell membranes by disrupting fluidity and permeability. They can also adversely effect the function of membrane bound proteins such as enzyme and receptors. (Abheri D.Sarma *et al*.,2010).

Free radical-targets

Free radicals attact three main cellular components

b) Lipid

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c) Protein

Direct damage to protein can be caused by ROS/ free radical can generated a range of stable as well as reactive product such as protein hydroperoxide that can generated additional radical particularly upon interaction with transition metal ions. This can affect many kinds of protein, interfering with enzyme activity and the function of structural proteins. (Abheri D.Sarma *et al*.,2010).

d) DNA

Fragmenting of DNA caused by free radical attack cause activation of the poly (ADP- ribose) synthetase enzyme. This split NAD+ to ai the repaire of DNA. However If, the damage is extensive, NAD+ level may become depleted to the extent that the cell may no longer be able to function and dies.This site of tissue damage by free radical is dependent on the tissue and the reactive species involved. Extensive damage can lead to the

death of the cell: this may be necrosis or apoptosis depending on type of cellular damage. When a cell membrane or an organelle membrane is damage by free radical, it loses it protective properties. This put the health of entire cell at risk.(Abheri D.Sarma *et al*.,2010).

Type of free radicals

Most free radicals are coming from oxygen atoms and are called Reactive Oxygen Species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen . It is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, DNA and other molecules. Our body can neutralize superoxide ions by producing superoxide.

1. Superoxide anion ($O2^-$)

One –electron reduction state of O2, formed in many autoxidation reaction and by the electron transport chain. Rather unreactive but can release Fe^{2+} from iron- sulfur proteins and ferritin. Undergoes dismutation to form H2O2 spontaneously or by enzyme catalysis and is precursor for metal-catalyzed OH formation.(V.Lobo *et al.,* 2010). In biological system superoxide ion ($O2^-$) is the most widespread ROS. It is formed by various enzymatic and non- enzymatic process. as well as a string nucleophile. It could participate in DNA methylation, histone methylation and acetylation through mechanism of nucleophilic substitution and free radical abstraction. The enzyme which generaye superoxide are xanthibne oxidase, lipoxygenase, COX and NADPH dependent oxidase.

It can present as $O2^-$ or hydroperoxyl radical at low pH. It has both reducing and oxidizing properties.(S.Bhiswas *et al*., 2010).

2. Hydrogen peroxide (H2O2)

It is not a free radical but it is involved in the production of many reactive oxygen species. Hydrogen peroxide is a by-product of oxygen metabolism and is neutralized by peroxidases. Sometimes reactive nitrogen atoms are involved and these free radicals grouped under Reactive Nitrogen Species (RNS). Nitric acid is the most important RNS. Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals. These metals do not have that strong electron affinity but can easily accept and donate electrons (A. Cumpustey et al., 2018).

3. Hydroxyl radical (OH)

It is formed by the reduction of an oxygen molecule in the electron transport chain. It is a neutral (not charged) form of the hydroxide ion. Hydroxyl radicals are highly reactive and form an important part of radical biochemistry. Unlike superoxide the hydroxyl radical cannot be eliminated by an enzymatic reaction. Is has a very short half-life and will only react with molecules its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins.

4. Singlet oxygen

Singlet oxygen is formed by our immune system. Singlet oxygen causes oxidation
.of LDL cholesterol

5. Organic hydroperoxide (ROOH)

Formed by radical reaction with cellular components such as lipid nucleobases.(V.Lobo *et al.,* 2010).

Roll of free radical in inflammation

Role of free radical in inflammatory reaction is well described. The free radical especially, The ROS create oxidative stress in the cell leading to inflammatory and infectious condition. Phagocytic cells including polymorphonuclear (neutrophills, eosinophills) and mononuclear cells(macrophages and lymphocytes) produce excessive amount of ROS which play an important role in the host defence mechanism.Beside their defensive effects these excessively produced ROSderegulate the cellular function causing cellular and tissue damage,which in turn augments the state of inflammation.(Shaikh R.U, Pund M.M. *et al*.,2015).

Reactive oxygen species (ROS)

In living system , among the radical species, oxygen derived radicals are most important and it is called as ROS. Reactive oxygen species (ROS) are very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROS is formed as a natural byproduct of the normal metabolism of oxygen and have

important roles in cell signaling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures. Platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. Generally, harmful effects of reactive oxygen species on the cell are most often like damage of DNA, oxidations of poly desaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors (A.K.Ghosh *et al*.,2010; Abheri D. Sarma *et al*.,2010).

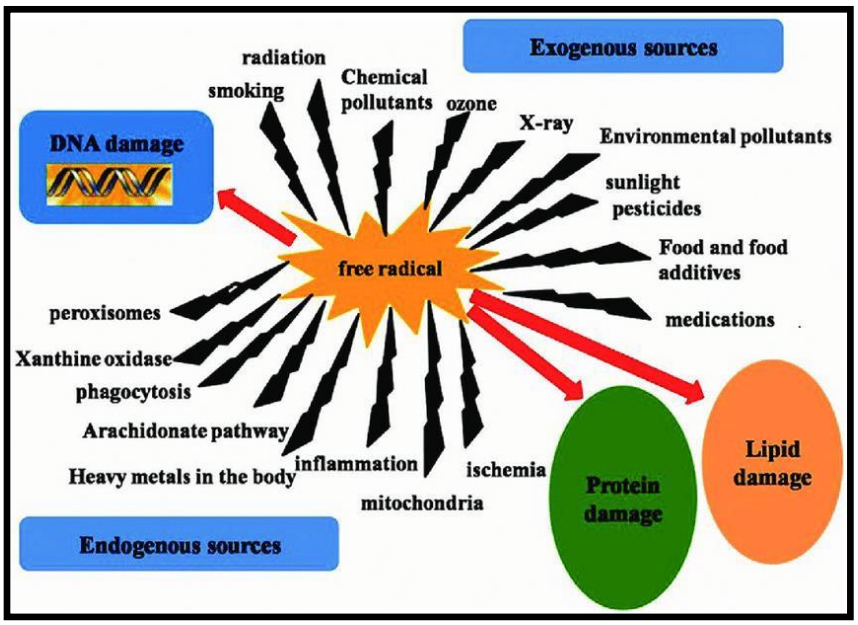


Fig No.3.2 Endogenous and Exogeneous source of ROS

ROS can produced from several sources –

1) **Endogenous source**, such as Xanthine oxidase, cytochrome oxidase(P450) metabolism cyclooxygenase mediated unsaturated fatty acid oxidation, oxidation of catecholamine, mitochondrial oxidation, inflammation, phagocytosis, ischemic reperfusion injury, exercise activation of leukocyte nicotinamide adenine dinucleotide phosphate oxidase, iron release, and reduction -oxidatiom reation cycling In eukaryotic cell, ROS is generated mostly in electron transport chain of mitochondria. (S.Biswas *et al*.,2017; K.V. Nagani *et al*.,2010).

2) **Exogenous source** like smoking of cigarette, X-ray exposure, industrial chemicalsorganic solvents like butylated hydroxytoulene, ozone, and air pollutants also up regulate ROS production(K.V. Nagani *et al*.,2010). The compounds are decomposed into ROS after they penetrate th body, The damaging effect of ROS on cellular macromolecules such as protein ,lipid,nucleic acid are causing alteration in protein ,and nucleic acid. Formation of these free radical leads to initiation and progression of many diseases such as diabetes, heart diseases, atheroscelerosis, liver diseases and cancer.(A.A.Adwas *et al.,* 2019).

Oxidative stress

Oxidative stress, defined as a **"**disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, leads to production of tissue damage to a wide range of molecular species including lipids, proteins and nucleic acid**"**. (P. Priya *et al.*,2014). In a normal helthy human body, the formation and detoxification favours an increases in ROS level, leading to disturbed cellular function. ROS causes damage to cellular macromolecules. Important free radicals are described and biological sources of origin discussed, together with the major antioxidant defense mechanisms. (A.A. Adwas *et al.,* 2019). The production of these reactive species occurs continuously in the organism; this production may be endogenous or exogenous. Some of these reactive species are generated as "chemical accidents", i.e. undesired secondary reactions between biomolecules or alternatively in the detoxification of xenobiotic (Devki *et al.,* 2017).

Fig: 3.3 Oxidative stress in human body

ANTI-OXIDANT
Definition

Antioxidant may be defined as **"**compound that inhibit or delay oxidation of the other molecules by inhibiting the initaiation or propagation of oxidizing chain reaction**"**(A. Jenitha *et al*.,2016)**.** Antioxidant can also protect the human body from free radicals and ROS and Preventt occurance diseases such as cancer, aging. It can interfere with oxidation process by reacting with free radical, chelating, catalytic metal, and also by acting as oxygen sacavengers. Antioxidants is found in dietary sources like fruits, vegetables and tea body cell from oxidative damage to a target molecule caused by oxidation , they protect from free radicles by ROS (A. Jenitha *et al*.,2016).

Free radical formation is controlled naturally by various beneficial compounds known as antioxidant. When availability of antioxidant is limited that this damage can become cumulative and debilitating. Free radicals are electrically charged molecules, i.e., the, have an unpaired electron , which causes them to seek out and captured electron from other substance in order to neutralize themselves. Althought the initial attack causes free radical to become neutralized, another free radical is formed in the process, causing chain reaction to occurs. And until free radical are deactivated, thousand of free radicals reactions can occurs within second of the initial reaction. Antioxidant are capable of stabilizing, or deactivating, free radicals before they attact on cells (A. Jenitha *et al*.,2016)**.**

Two principle mechanism of action have been proposed for antioxidants.

1. Chain- breaking mechanism by which primary antioxidant donate an electron to the free radical present in systems.
2. Removal of ROS initiator by quenching chain- initiating catalyst. Antioxidant may exert their effect on biological systems by different mechanism including electron donation, metal ion chelation, co-antioxidant, or by gene expression regulation.(V.Lobo *et al.*, 2010).

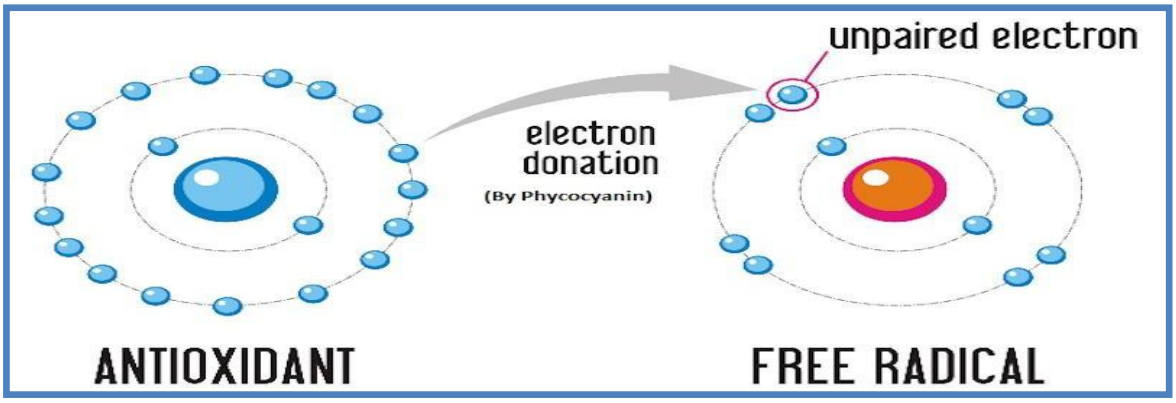


Fig . 3.4 Mechanism of antioxidant

Type of antioxidants

Antioxidant act as radical scavenger ,hydrogen donor, electron donar, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, metal chelating agents. Both enzymatic and non- enxymatic antioxidant exist in the intracellular and extracellular environment to detoxify ROS (V. Lobo *et al.*, 2010).

1) Natural Antioxidant (Exogenous)
2) Synthetic Antioxidant (Endogenous)

Medicinal plant contain many antioxidant such as Vitamine (A,C,E,K), carotenoid, flavonoid (Flavons, isoflavons, catechine,,isocatechine), polyphienol (ellagic acid, gallic acid, tannin), saponine, enzymes, and minerals (copper, selenium, iodine, zinc)(S.V. Chanda *et al.*,2010).

1) Natural antioxidant

(Exogeneous) Natural antioxidant tends to be safer and also posses anti-viral, anti- fungal, anti-inflammatory, anti-cancer, anti-mutagenic, anti-tumour, hepatoprotective properties. The source of natural antioxidant may be all or any part of plant such as fruits, peels, seeds, leaves, barks, routes, peels, nuts, vegetables, catalase, superoxide dismutase (SOD), dietary glutathione, plants etc.(K.V.Nagani *et al.*,2010).

2) Synthetic antioxidant

(Endogenous) Synthetic antioxidant are chemically synthesized compounds they do not occure in nature and added to food as preservative to help prevent lipid oxidation. Due to the inherent instability of natural antioxidant . Synthetic antioxidant used to stabilize fats and oils. Butylated hydroxyanisole (BHA), Butylated hydroxytoluene(BHT) were originally developed to protect petroleum from oxidative gumming(E. M. Atta *et al.*,2018; S.V. Chanda *et al.*,2010).

The use of natural antioxidants in food, cosmetic and therapeutic industry would be promising alternative for synthetic antioxidants in respect of low cost, highly compatible with dietary intake and no harmful effects inside the human body. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers (Brown *et al.*, 1998).

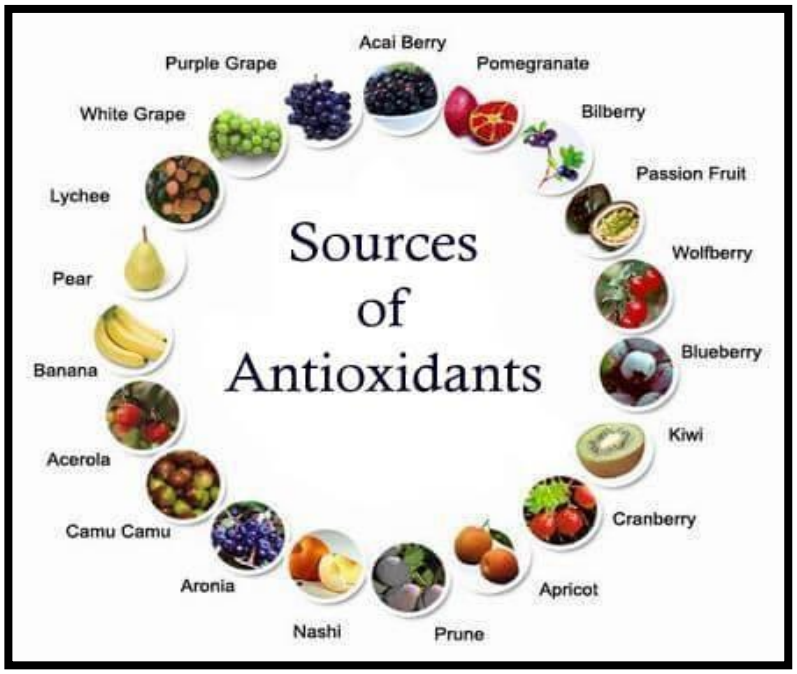


Fig : 3.5 Source of Antioxidant

Plant with anti-inflammatory and analgesic potential

1) ***MANILKARA ZAPOTA (L.) P. ROYEN (SAPOTACEA)***

Treatment with both low dose 200mg/kg b.wt and high dose 400 mg/kg b. wt of herbal ethanolic extract of dried powder of Manilkara zapota linn increased the reaction time in the method (eddy's hot plate method , acetic acid induced writhing)confirming analgesic activity and similarity anti-inflammatory activity (carragenan and histamine induced paw odema) shown significant increase in % protection against paw volume . However, comparatively 400mg/kg b.wt dose of Manilkara zapota linn is more efficacious.(Ayesha k. *et al*.,2016).

2) ***MANILKARA ZAPOTA (L.) P. WHOLE FRUIT ROYEN (SAPOTACEA)***

leaves carried out analgesic activity of petroleum ether and ethanolic extract of Manilkara zapota on laboratory animals,using Hot plate method. The result of present study concluded that petroleum ether and ethanolic extract of Manilkara zapota at Dose of *200 mg/kg* posseses analgesic effects ,which is in accordance with its experimental studies.(Pankaj K jain *et al*.,2011).

3) ***CURCUMA LONGA***

Curcuma longa (common name is Turmeric) is an Indian indigenous plant. The most important secondary metabolite of Curcuma longa is curcumin, which is responsible for anti- inflammatory effect of this plant. Many clinical trials have been done for proving the anti- inflammatory effect of curcumin. Their results suggest that curcumin can be effective in improving inflammation of rheumatoid arthritis and reducing clinical manifestation of rheumatoid arthritis, such as joint swelling and morning stiffness in comparison with phenyl butazone which is used as a positive control. Also, curcumin was tested in patients with anterior uveitis; after 2 weeks, exhaustive remission occurred (Holt *et al.,* 2005). The effectiveness of curcumin in patients with dyspepsia and/or gastric ulcer was proved by another clinical trial. In this study, subjects experienced remission after 12 weeks (maximum). Curcumin is beneficial in irritable bowel syndrome (IBS) treatment and also works as a reducing agent to delayed graft rejection (DGR) after kidney transplant surgery. Curcumin

likewise has a beneficial effect in inhibition of inflammatory bowel disease (IBD) and reduction in sedimentation rate in patientswho suffered from IBD. It is also proven to be beneficial in maintaining amelioration of ulcerative colitis and psoriasis (by the selective prohibition of phosphorylase kinase) (Bagad *et al*., 2013; Jurenka *et al*., 2009).

4) *URTICA DIOICA (URTICACEAE)*

Urtica dioica (common name is stinging nettle) is a member of Urticaceae family. Nettle leaf has been investigated to prove its anti-inflammatory effect in a pilot study. 50mg Diclofenac per day was administered to patients with acute arthritis together with 50 mg infusion of Urtica dioica orally. This remedy has caused remarkable attenuation in CRP level and some patients' complaints for 200 mg Diclofenac per day; according to these outcomes, Urtica dioica when combined with NSAIDs have an outstanding synergistic effect (Randall et al., 2000). Topical effectiveness of nettle leaf has been assessed in osteoarthritis of thumb through RCT; significant alleviation in pain, stiffness, and anti-inflammatory and analgesic therapy requirements has been observed. The combination of nettle leaf with rosehip and willow bark has suppressed IL-1β and COX- 2 in chondrocytes. In this in vitro study, chondroprotective and anti-inflammatory effects of this botanical extract have been proved. Leaf extract of Urtica dioica has had inhibitory potential on proinflammatory transcription factor NF-κB (scientific studies have shown elevation in NF-κB in synovial fluid of rheumatoid arthritis patients). This extract has had anti-inflammatory potential in allergic rhinitis by the following pathways: antagonizing H1-receptor, reducing of PGD2 production (allergy specific prostaglandin), and inhibitory effect on mast cell tryptase (Roschek et al., 2009; Johnsonet al., 2013).

5) *ZINGIBER OFFICINALE*

Zingiber officinale (common name is ginger) is a native plant from south-east Asia. Oral administration of Zingiber officinale extract has shown different and inconsistent effects, depending on the quantity of consumption. Although administration of squeezed ginger extract to mice one time or twice has elevated the tumor necrosis factor-α (TNF-α) in peritoneal cells, long-term consumption of the extract has increased the serum corticosterone level and has reduced proinflammatory markers (Haghighi et al., 2006). Zingiber officinale was also tested in type 2 diabetic patients with low-grade inflammation; after 2 months of treatment, serum level of TNF-α and high-sensitivity C-reactive protein (hs-CRP) were decreased definitely. In patients with osteoarthritis, ginger had not only efficacy in pain improvement identical to Diclofenac but also no side effects. Ginger extract has been compared to Ibuprofen and Indomethacin in osteoarthritis patients; the results have exerted improving function of Ibuprofen, Indomethacin, and ginger extract equally in pain score. Ginger powder has had ameliorative effect in musculoskeletal and rheumatism patients through inhibiting cyclooxygenase and lipooxygenase pathway in synovial fluid (Mahluji *et al*., 2013; Khalvat *et al*., 2005).

6) *BRYOPHYLLUM PINNATUM (CRASSULACEAE)*

The anti-inflammatory potential of *Bryophyllum pinnatum* was investigated by ojewole et al. The study was undertaken to investigate anti- inflammatory and of the plant leaf aqueous extract in experimental animal models. In this experiment using fresh egg albumin- induced pedal (paw) oedema model and drug taken Diclofenac 100 mg/kg. The results revealed of this experimental animal study suggest that *Bryophyllum pinnatum* leaf aqueous extract possessed anti-inflammatory (John A.O *et al.*,2005).

7) *ALBIZIAL LEBBECK (MIMOSAEAE)*

The bark extract of *Albizial Lebbeck* obtained by cold extraction of mixture of equal proportions of petroleum ether, ethyl acetate and methanol was chosen for pharmacological screening. In rat paw edema model induced by carrageenan, the extract at the 200 and 400 mg/kg dose level showed 27.51% and 36.68% ($P < 0.001$) inhibition of edema volume at the end of 4 h. Present study on extract of *A. lebbeck* has demonstrated that this plant has significant analgesic and anti-inflammatory properties, and it justifies the traditional use of this plant in the treatment of various type of pains and inflammation. (Saha A *et al 2010*).

8) *CASSIA OCCIDENTALIS (CAESALPINIAXEAE)*

Sreejith et al. was evaluated anti-inflammatory potential of whole plant of *Cassia occidentalis* using ethanolic extract. For investigation of anti- inflammatory potential dose taken 250 mg/kg and using carrageenan induced paw edema model. The result revealed that

significant reduction in malondialdehyde levels of murine hepatic microsomes and significantly reduced carrageenan induced inflammation in mice at a dose of 250 mg/kg (Saha A *et al 2010*).

9) ***MANILKARA ZAPOTA (L.) P. ROYEN (SAPOTACEA)***

fruit bark carried out Evaluation of Anti- inflammatory Activity and Total Flavonoids of *Manilkarazapota* (Linn.) Bark, The crude methanolic extract of the bark of *Manilkarazapota*. The anti-inflammatory activity was studied using carrageenan and histamine-induced rat paw edema test at different doses (200 and 400 mg/kg body weight) of the methanol extract. At the dose of 400 mg/kg body weight, the extract showed a significant anti- inflammatory activity both in the carrageenan and histamine-induced oedema test models in rats showing 59.72% and 60.0% reduction in the paw volume comparable ($P<0.01$) to that produced by the standard drug indomethacin (62.50% and 65.16%) . (Hossain *et al,* (2012).

10) ***AEGLE MARMELOS (RUTACEAE)***

The aqueous extract of the root bark of Bilwa was prepared and tested for anti- inflammatory activity in albino rats using Carrageenan induced paw edema model and cotton pellet induced granuloma and the standard drug was taken indomethacin and Bilwa. The result revealed that anti-inflammatory activity was expressed the inhibition (Benni *et al.*, 2011).

11) ***SALVIA OFFICINALIS (LAMIACEAE)***

Salvia officinalis (commonly known as) is a member of Lamiaceae family. Carnosol and carnosic acid are phenolic diterpenes which have had anti-inflammatory activity. These two components could have inhibited PGE2 production via microsomal PGE2 synthase-1 inhibition. Chloroform extract of sage leaves has shown atopic anti-inflammatory effect in mice. However, sage essential oil has not shown any immune modulatory effect in mice which had underwent cyclophosphamide- mediated immunosuppression. It is also worth mentioning that have reported generalized tonic-clonic seizures following accidental exposure to sage oil in a newborn and a child (Rodrigues *et al.,* 2012; Baricevic *et al.,* 2001).

12) ***ELAEAGNUS ANGUSTIFOLIA***

Elaeagnus angustifolia (common name is Oleaster) is a member of Elaeagnaceae family. The effectiveness of Oleaster in the treatment of oral lichen planus (OLP) lesion has been evaluated in an RCT with 28 patients. Seventy five percent and 50–75%attenuation in pain and lesion size, respectively, have been observed in the case group. In another randomized clinical trial which has been carried out on 90 knee osteoarthritis female patients, a significant attenuation in TNF-α and matrix metalloprotein- 1 (MMP-1) (pro-inflammatory mediators) and alleviation in IL-10 (an anti-inflammatory cytokine) have been reported in active therapy group. Oleaster extract has demonstrated an anti-inflammatory effect in an animal model but this effect was not significant in comparison with sodium salicylate. Aqueous extract of this fruit has shown anti-inflammatory properties in mice through COX-1 and COX-2 inhibition; the evidence has exerted no correlation between corticosterone level and that of anti-inflammatory action (Ahmadiani *et al*., 2000; Farahbakhsh *et al*., 2011).

13) ***NELUMBO NUCIFERA***

N. nucifera, also known as Indian lotus, belongs to family *Nelumbonaceae*. This plant is an aquatic perennial. Under favorable circumstances, its seeds may remain viable for many years, with the oldest recorded lotus germination being from that of seeds 1300 years old recovered from a dry lakebed in Northeastern China. Phytochemicals include flavanol miquelianin as well as alkaloids and also nuciferine and aporphine. In *N. nucifer*a Lotus flower tea is used in Chinese medicine as an analgesic and for its sedating and antidepressant properties. Essential oils containing menthol or 1,8-cineol have cooling properties that can directly numb the pain and discomfort of a headache. Essential oils of this plant with hormone-balancing properties, such as lavender and clary sage, may be especially effective at reducing headaches caused by hormonal imbalances during a woman's menstrual cycle.(Nandini S.*et al.*, 2018).

14) ***ADATHOA VASICA***

A. vasica (Malabar nut tree) belongs to *Acanthaceae* plant family. It is a small evergreen, sub-herbaceous bush which grows commonly in open plains, especially in the Lower Himalayas. The phytochemicals include alkaloids such as vasicine and vasicinone, essential

oil, and an organic acid called adathoa vasica. It is a highly reputed plant used in the Ayurvedic system of medicine for the treatment of various ailments of respiratory systems such as bronchitis and asthma, and it is also used in the treatment of malaria, dysentery, and diarrhea[9] and also has anti-inflammatory, analgesic, diarrhea, dysentery, antioxidant, hepatoprotective, sedative, antispasmodic, and anthelmintic properties.[10] The administration ofethanolic extract of the root of *A. vasica* at the doses of 200 and 400 mg/kg and morphine (10 mg/kg), a reference drug, significantly raised the pain threshold at observation time of 45 min in comparison with control ($P < 0.001$). Oral administration of the ethanolic extract of *A. vasica* significantly reduced the number of writhings induced by acetic acid in rat..(Nandini S.*et al.,* 2018).

15) *GUIERA SENEGALENSIS GMEL*

G. senegalensis belongs to *Combretaceae* family, which is a herb of a wide range of geographical distribution in Africa, starting from rainforest region of Nigeria to the arid zone areas of Mali.[8,13] It is a common herbal, antimalarial, and antipyretic drug. Phytochemicals include anthraquinones, flavonoid, tannin, and saponin. This plant is known for is antibacterial and analgesic property. This is a medicinal plant whose aqueous decoction extracts are used as an analgesic. The aqueous methanol extracts at the various doses tested afforded varying protection against thermal stimulus in mice. However, AMRBE significantly ($P < 0.05$) increased the mean latency of pain response. The standard agent, morphine, afforded more than 400% protection against thermal stimulus. .(Nandini S.*et al.,* 2018).

16) *MENTHA PIPERITA*

M. piperita (peppermint) belongs to *Labiatae* family. It is a hybrid mint, a cross between watermint and spearmint. The plant, indigenous to Europe and the Middle East, is now widespread in cultivation in many regions of the world. It is a herbaceous rhizomatous perennialplantgrowingto 30–90cm (12–35 in) tall, with smooth stems, square in cross-section. It is found wild occasionally with its parent species.[6,7] Phytochemical constituents include volatile oils (menthol, menthone, and methyl salicylate), flavonoids (methoxide and rutin), and carotenes (tannins and choline). It has also been shown to be a safe and effective short- term treatment for irritable bowel syndrome. According to Commission E, peppermint oil may also act as a carminative, cholagouge, antibacterial, and secretolytic, and it has a cooling action.[8] Peppermint oil is excellent for mental fatigue and depression, refreshing the spirit, stimulating mental agility, and improving concentration. Herbalists consider peppermint an astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue. The aqueous extract of *M. piperita* leaf, at the i.p doses 200 and 400 mg/kg, showed significant analgesic effects against both acetic acid-induced writhing and hot plate- induced thermal stimulation in mice, with protection values of 51.79% and 20.21%. Topically, *M. piperita* essential oil is employed as an analgesic compound for diseases of the pharynx and in the relief of tension headache and migraines. .(Nandini S.*et al.,* 2018).

17) *GLYCYRRHIZA GLABRA*

G. glabra root (rhizome) includes glycerine which is 50 times more than sucrose. Its commercial extracts include glycyrrhizin in ammonium salt and *G. glabra* alcoholic extract which comprises of four active ingredients: Glycemia coumarin glycerine, hydro glia aspirin C, and dehydrogol aspirin D. Other ingredients of this herb are flavonoids including isoflavone, liquiritin, isoliquiritin, formononetin polysaccharides, esteroles, coumarins, asparagine, amino acids, resin, starch, oil essences, and saponins. This herb is a remedy for coughing; it has mucolytic, anti-inflammatory, and laxative properties and is used effectively to treat stomach and duodenum illnesses. It is also used in treating upper respiratory tract infections, bronchitis, peptic ulcers, duodenal ulcers, chronic gastritis, rheumatism, arthritis, and adrenal. Its products are widely used in pharmaceuticals as sweetener and binder. Liquorice is useful in treating skin complications such as dermatitis, eczema, and pruritus. It has anti-infectious, antiseptic, antibacterial, anti-hepatotoxicity, antivirus, and antiphlogistic characteristics. Liquorice causes antispasmodic effects in GI tract and visceral pain relief through inhibiting phosphodiesterase.[14,15] Its hydroalcoholic extract through increasing defensive factors of gastric mucosa induces anti-ulcer mechanisms. Glycyrrhizin is another one of the liquorice ingredients. Its oral use inhibits 11-beta dehydrogenase enzyme and consequently increases the blood cortisol level. It is probable that this ingredient reduces pain through reducing inflammation..(Nandini S.*et al.,* 2018).

3. AIM AND OBJECTIVE

Aim

The aim of the present work was to carry out pharmacological investigation of selected plant part extract for anti-inflammatory and analgesic activity in experimental animals. The reported plant part *manilkara zapota (L.) P. Royen* fruit peel was selected from extensive literature survey of plants mentioned for the treatment of analgesic and anti-inflammatory activity in traditional system of Indian medicine. Based on the review of literature, reported pharmacological activity, chemical constituents and traditional claim was selected for the study.

Aim: Pharmacological Evaluation of *Manilkara zapota (L.) P. Royen* fruit peel extract for Anti-inflammatory and analgesic effects in experimental animals.

Objective:

Literature survey reveals that the several plants have always been proven as a better source of drugs when it comes to treatment of Anti-inflammatory and analgesic and its complication.In literature study for my presented thesis it reveals that fruit peels of *Manilkara zapota (L.) P. Royen* has several phytoconstituents which shows Antiinflamatory and analgesic activity which could pharmacologically be evaluated for this activity. Hence, it was thought worth to undertake present study to screen Anti- inflammatory and analgesic effects of *Manilkara zapota (L.) P. Royen* fruit peel extract .
The Broader objective for studies are as follows:

- To collect, identify and authenticate and evaluate fruit peel of *Manilkara zapota (L.)P. Royen* for effective control of Anti-inflammatory and analgesic activity.
- To prepare extracts of fruit peel depending on its polarity using proper solvent.
- To use the current scientific literature to identify potential target compound .
- To carry out preliminary qualitative phytochemical analysis of the the *Manilkara zapota (L.) P. Royen* fruit peel extract.
- To Evaluate Anti-oxidant activity of *Manilkara zapota (L.) P. Royen* fruit peel extract using DPPH radical scavenging Assay.
- To Evaluate Heat induced hemolysis effect and inhibition of albumin denaturation effect of *Manilkara zapota (L.) P. Royen* fruit peel extract by using *In-vitro* anti- inflammatory model
- To.Evaluate or Investigate anti-inflammatory activity of the *Manilkara zapota (L.) P. Royen* fruit peel extact in wistar rats by using Carragenan induced paw edema.
- To investigate analgesic activity of the *Manilkara zapota (L.) P. Royen* fruit peel extract in Mice by using Tail flick test and Hot plate test .
- To interpret the obtained data statistically.
- To disseminate of the research findings by publications for the health care providers and lay public.

4. NEED FOR STUDY

India is a rich source of medicinal palnts and a number of plant derived oils and extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Sidhha. Only a few of them have been scientifically explored. Plant derived natural product such as phenol ,flavonoids, terpenes, saponin , glycosides gallic acid, quercetin, and alkaloids have received considerable attention in recent years due to their diverse pharmacological properties including inflammatory, antipyretic analgesic, anti- oxidant activities. (S. Shukla; A. Mehta *et al*., 2009).

Medicinal plants play an important role in the development of potent therapeutic agents. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. Herbal drugs are use of therapeutic herbs to prevent and treat diseases ,disorder and ailments or to support health and healing. These are drugs or preparations made from a plant or plants and used for any of such purposes. Herbal drugs are the oldest form of health care known to mankind.(K. Pathak; R. J. Das *et al*.,2013).

World Health Organization has set precise guidelines for the evaluation of the safety, efficacy, and quality of herbal medicines. They have inexpensive, easily and abundantly available, also with less side effects, low minium cost, complete accessesibility, more protection, potency and efficiency is very high. Herbal drug is a chief constituent in traditional medicine and a common constituent in ayurvedic, homeopathic, naturopathic and other medicine systems. Herbs are usually considered as safe since they belong to natural sources . The use of herbal drugs due to toxicity and side effects of allopathic medicines, has led to rapid increase in the number of herbal drug manufacturers. For the past few decades, herbal drugs have been more and more consumed by the people with no prescription.(K. Pathak; R. J. Das *et al*.,2013).

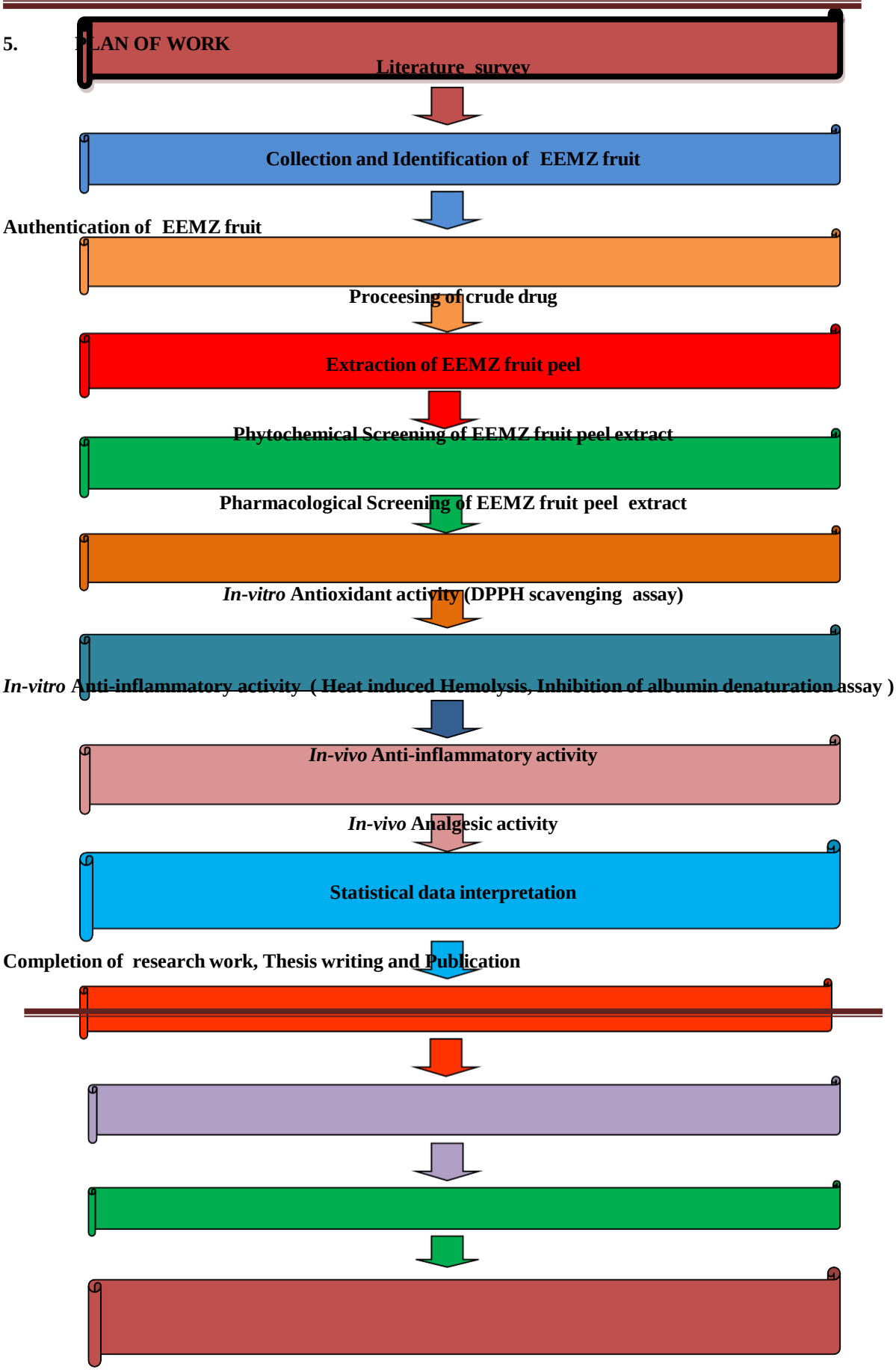
Seed, leaves, peels, stems, bark, roots, flowers, and extract of all of these have been used in herbal drugs. Herbal product have reached extensive adequacy as beneficial agents like antimicrobial, analgesic, sedative, antioxidant, anti-inflammatory, antidiabetic activities.(B. Maiti; Nagori B.P *et al*.,2011).

In acute and chronic inflammation NSAIDs drug are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain., fever, inflammation and rheumatic disorders. However, their use is associated with adverse effects at the level of digestive tract ,ranging from dyspeptic symptoms, GIT erosions and peptic ulcer to more serious complication, such as over bleeding. (Md.H.Hossain; F.Jahan *et al*.,2012). Therefore to overcome the toxicity of NSAIDs, the development of new anti-inflammatory drugs is still necessary and the natural product such as medicinal plants could lead in discovering new anti-inflammatory drugs with less undesirable effects. Now-a- days attention is being focused in the investigation of the efficacy of plat based drugs used in the traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies. (Md.H.Hossain; F.Jahan *et al*.,2012)

Manilkara zapota is a member of the family of *Sapotaceae*. Pharmacologically, it has been documented to posses Antioxidant and Antimicrobial. The Present study was explore the Pharmacological evaluation of *Manilkara zapota (L) P. Royen* fruit peel extract of potential for Anti-inflammatory and analgesic activity in animal model. Previous study reveled several certain polyphenols in *Manilkara zapota (L) P. Royen* fruit peel corresponding to the higest level of free radical scavenging effects. Studies have shown that the main constituent of fruit peel are tannin, saponin, toatal phenolic content, quercetin, epicatechin, catechin, gallo-catechin like phytochemicals, Thus these identified Anti-oxidant and other phytoconstituent in *Manilkara zapota (L)* fruit peel needs to be evaluated for Anti-inflammatory and analgesic effects.(Karle p.*et al*.,2018 and Gomathi *et al*.,2013)

Althought lots of works has been done on the *Manilkara zapota* fruits Leaves, bark, seed and whole fruit like analgesic and anti-inflammatory activity but anti- inflammatory and analgesic activity does not performed on the peels of *Manilkara zapota* fruit. The leaves, bark and whole fruit total contain phenolic content, Flavonoid, Tannin, Saponin, carbohydrates, proteins this phytoconstituents exhibit the antiinflammatory, analgesic and antioxidant activity. Total phenolic content and Flavonoid ,Tannin, saponin phytoconstituent are also present in this fruit peels . It is the claim of my Research work proposal as per literature survey hence, selected plant part (PEEL) of *Manilkara zapota (L.) P.Royen* fruit peel needs to be evaluated. (Karle P.*et al* 2019).

5. PLAN OF WORK



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6. MATERIAL AND METHODS

Materials:

Table .6.1: List of Drug and chemicals

Sr. No	Drugs and Chemicals	Manufacturer
1.	Ethanol 80%	HiMedia Laboratories Pvt Ltd, Mumbai
2.	DPPH	HiMedia Laboratories Pvt Ltd,Mu mbai
3.	Carrageenan	HiMedia Laboratories Pvt Ltd, Mumbai
4.	Ascorbic acid	HiMedia Laboratories Pvt Ltd, Mumbai
5.	Dragendorff's regent	Loba Chemie Pvt. Ltd. Mumbai
6.	Hydroclric acid	Loba Chemie Pvt. Ltd. Mumbai
7.	Wagner's reagent	Loba Chemie Pvt. Ltd. Mumbai
8.	Mayer's reagent	Loba Chemie Pvt. Ltd. Mumbai
9.	Hager's reagent	Loba Chemie Pvt. Ltd. Mumbai
10.	Ferric chloride	Loba Chemie Pvt. Ltd. Mumbai
11.	Sulphuric acid	Fine Chem Industries, Mumbai
12.	Chloroform, NAOH	Fine Chem Industries, Mumbai
13.	Glacial acetic acid	Fine Chem Industries, Mumbai
14.	Benedicts reagent	Loba Chemie Pvt. Ltd. Mumbai
15.	Hydrochloric acid	Loba Chemie Pvt. Ltd. Mumbai
16.	Petroleum ether	Loba Chemie Pvt. Ltd. Mumbai
17.	Diclofenac	Shipla ,Mumabi
18.	EEMZ	Nanded city, Maharashtra India
19.	Alpha naphthoal	Loba Chemie Pvt. Ltd. Mumbai
20.	Phosphate buffered	Loba Chemie Pvt. Ltd. Mumbai

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Table .6.2: List of Instruments and apparatus

Sr.No	Instruments/Apparatus	Manufacturer
1.	Soxhlet apparatus	Jiangsu sanaisi scientific instrument co, Ltd.
2.	Laboratory centrifuge	Sukhras Machine Pvt. Ltd, Mumbai
3.	UV Spectrophotometer	Shimadzu (Asia Pacific) Pvt Ltd, Tamil Nadu
4.	Plethysmometer	Orchid Scientific India Pvt Ltd Nashik
5.	Dessicator	Garg Process glass India Pvt Ltd, Mumbai
6.	Analytical weighing balance	Sony electronic scale, Mumbai
7.	Rota evapourator	Sukhras Machine Pvt. Ltd, Mumbai

Extraction of plant material

Collection of plant material:

Fruits of *Manilkara zapota (L.) P. Royen fruit* (Family sapotaceae) was Collected in the month of September 2019 from the local market of Nanded district city, Maharashtra state.

Identification and authentication of plant material

Fruit peels of *Manilkara zapota (L.) P.Royen* was identified and authenticated by Dr. Vishal R. Marathe, (plant taxonomy research Lab.) Dep. of Botany, N.E.S Science College, Nanded Maharashtra india as *Manilkara zapota (L.) P. Royen* fruit belonging to family (Sapotaceae).

Selection of solvent and extraction method

According to nature of Phytochemical present in plant extract such as flavonoids, gallic acid, catechin, quercetin, phenolic content and literature review, solvent and extraction method was selected.

HOT METHOD OF EXTRACTION- Extraction was done using soxhlet apparatus. Ethanol (80%) were used as solvent for the extraction.

Procedure:- Hot extract was prepared by using soxhlet extraction process. About 200 gram of powder extracted with 1L of solvent (ethanol 80%) at 40^0C for 24 Hours. Then the filtered out by using whatman filter paper No.1.The solvent containing active constituent was transferred to rota evapourator to evaporate the solvent and to get solid extract. Then they was defatted with petroleum ether for removing the fats from extract. The extract was kept in deep freez at 4^0C for further study. (Gahlol M, Bhatt P, Joshi J. *et al*.,2018). Soxhlet apparatus were use of (1000ml capacity).

Preparation for extraction

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Fig : 6.1 Assembly setup for soxhlet extraction of *(Manilkara zapota fruit peel)*

After the collection of fruits, Fruit peels were thoroughly washout under tap water, then dried 10-15 days in room temperature. Peels powdered coarsely using electric grinder. 100 grams dried powder was extracted with 80% ethanol by using Soxhlet apparatus. The ethanolic extract of *Manilkara zapota* fruit peel obtained and the dried mass was weighed and recorded.

$$(\%)\ \text{Yield} = \frac{\text{Wt. of Extract}}{\text{Wt. of powdered drug}} \times 100$$

The % yield of *Manilkara zapota* fruit peel was found to be ……**3.45 % w/w.**

Experimental animals

Rat

Fig: 6.2 Rat

Albino Wistar rats of either sexes (100-150 g) were used for the study they are obtained from animal house of Sudhakarrao Naik Institute of pharmacy, Pusad Maharashtra. All the rats were keep At temp. of 24+-2^{0}C , 30-60 % humidity in polypropylene rat cages with stainlesssteel coverlids and wheat straw were used as bedding material. The animals were facilitated withstandard environmental condition of period (12:12 h dark: light cycle) for 1 week for acclimation. Fed with standard rodent diet with water *ad libitum*. (Meshram *et al.*,2016). Institutional animal ethical committee **(Reg.No.729/PO/Re/S/11/CPCSEA).**

Mice:

Fig: 6.3 Mice

The 6-8 week of Swiss albino mice of either sexes (25-35g) were used for the study. All the mice were keep At.

temp. of 25 +-2^{0}C and 70-80 % humidity in standard plastic mice cages with stainless steel coverlids and wheat straw were used as bedding material. The animals were facilitated with standard environmental condition of period is (12:12 h dark: light cycle) for 1 were acclimation. Fed with standard diet and water *ad libitum*. *(* Sook-Ha-fan *et al.*,2014; P. Malleshappa; R.Chapeyil; *et al.*, 2018;A. Alemu *et al*., 2018).

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Approval of research protocol

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals .**(Reg.No.729/PO/Re/S/11/CPCSEA)** .

Selection of dose and Calculation

1) Determination of oral LD_{50} (lethal dose) as per OECD guidline 423 acute oral toxicity class .

2) Calculate for low, moderate and higher dose i.e. safe dose or effective dose as per OECD Guidelines 423.

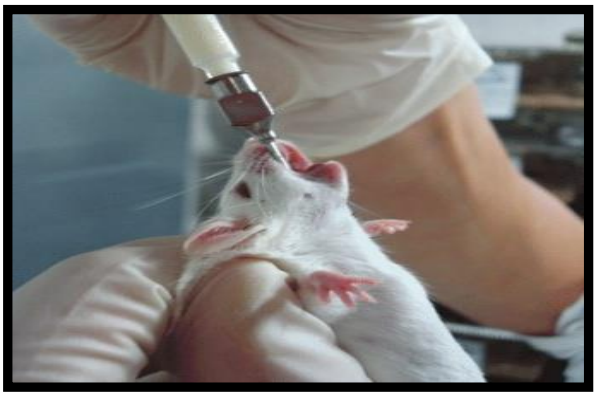

Fig: 6.4 Oral dosing of animal

Procedure :

Performed acute toxicity study on Ethanolic extract of *Manilkara zapota (L.) P. Royen* Fruit peel and found the oral LD_{50} was greater than 2000mg/kg b.wt for animals. Hence the above reference the dose of *Manilkara zapota (L.)* Royen fruit selected as 100 mg/kg, 200mg/kg, and 400mg/kg was selected for this study (Ayesha *et al., 2016* and Hossain *et al.,* 2012).

Preliminary Phytochemical Analysis

Qualitative chemical tests were carried out for the extracts to identify the presence of various chemical constituents present in the extract by using standard phytochemical procedure (Table 6.3) *(*Khandelwal *et al*., 2010; Jaradat N. *et al*., 2015).

Table 6.3:Preliminary Phytochemical test carried out for the EEMZ

Sr. No	Chemical test
1.	**Carbohydrate** 1 Molish test 2 Benedict test

2.	**Alkaloids** 1. Dragendoff's test 2. Mayer's test 3. Wagner's test
3.	**Glycoside** 1. Borntrager's test 2. Killer Killani test
4.	**Flavonoid** 1. Shinoda test 2. Alkaline reagent test
5.	**Tannins and phenol** 1. Fecl3 test 2. Lead acetate test
6.	**Amino acids** 1. Million's test 2. Ninhydrine test

Chemical test for detection of organic constituents.

1) Test for Carbohydrate Molish Test

To 2-3 ml aqueous extract , add few drops of alpha-naphthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids. (Khandelwal K.R. *et al*., 2010).

Benedict Test

Boil 2 ml of benedicts reagent with a extract, a reddish brown colour indicated the presences of the carbohydrates (Khandelwal K.R. *et al*., 2010).

2) Test for Alkaloid Dragenodroffs Test

2-3 ml Dragenodroffs reagent (Potassium iodide+ bismuth nitrate) added in to the extract which gives orange colour that indicted the presence of alkaloids**.** (Khandelwal K.R. *et al*., 2010).

Mayers Test

2-3 ml Potassium mercurc- iodide solution added in to the extract which gives yellow precipitate that indicatrd that alkaloid present. (Khandelwal K.R. *et al*., 2010).

Wagners Test

2-3 ml Iodine solution added in to extract which gives brown precipitate that indicted that the presence of alkaloids. (Khandelwal K.R. *et al*., 2010).

3) Test for Glycoside Bontrager Test

prepare aqueous solution of extract. Dil. H_2SO_4, chloroform and ammonia added into 3 ml aqueous solution

which gives pink –red colour that indicate the presence of glycosides. (Jaradat N. *et al*., 2015).

Keller-killiani Test

A mixture of acetic acid glacial 2ml with 2 drop of 2% FeCl3 solution was adeed to the extract and H2SO4 conc. A brown ring produced between the layers which indicated the entity of cardiac steroidal glycosides. (Jaradat N. *et al*., 2015).

4) Test for Flavonoid Shinoda Test

Piece of magneshium ribbon and HCL conc. Were mixed with extract after few minute pink coloured scarlet appeared that indictaed that the presence of flavonoids. (Jaradat N. *et al*., 2015.)

Alkaline reagent test
2 ml of 2% of NaOH solution mixed with extract, intensive yellow colour was formed , which turn into colourless when added 2 drop of diluted acid to solution, result indicted the presence of flavonoids.

5) Test for Tannin and phenol FeCl3
2 ml of 5 % of solution of FeCl3 mixed with extract. Black or blue green colour indicated that the presence of tannin and phenol. (Khandelwal K.R. *et al*., 2010).
Lead acetate test
2 ml of lead acetate solution with extract which gives white precipitate indicated that the presence of tannin or phenol**.** (Khandelwal K.R. *et al*., 2010).

6) Test for Amino acid Millons Test
2 ml of millons reagent mixed with extract , appeared white precipitate which upon gentle heating turned into red colour which indicted that presence of amino acid and proteins in plants. (Jaradat N. *et al*., 2015.)
Ninhydrin test
Boil 2ml of 0.2% ninhydrin solution with extract , appeared violet colour indice the presence of protein and amino acids. (Jaradat N. *et al*., 2015.)

Pharmacological screening of Ethanolic extract of *Manilkara zapota (L.) P. Royen* fruit peel.

***In-vitro* Study**

a) *In-vitro* Antioxidant study
In- vitro Antioxidant study of *Manilkara zapota (L.) P. Royen* fruit peel extract was carried out by using DPPH (1, 1-diphenyl-2- picryl hydrazyl) radical scavenging activity.DPPH free scavenging activity

Principle:
The free radical scavenging activity of the compound was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Free radical conta ins an odd electron, which absorb the UV light at wavelength 517 nm and also for visible deep blue color. This method involves the measurement of decrease in absorbance of DPPH, its maximum absorption at 517 nm. It is directly proportional to the concentration of the free radical scavenger added to DPPH reagent, disappearance of DPPH in test sample due to which purple solution changes to yellow.
The degree of discoloration indicated the scavenging potential of the antioxidant compounds in the term of hydrogen donating ability. The free radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2- picrylhydrazyl) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it was reduced. The changes in color (from deep violet to light yellow) were measured at 517 nm on a UV-visible light spectrophotometer. (Bogani *et al.,* 2007; Navghare V.V.,Dhawale .S.C. *et al*., 2016; chanda SV. *et al*.,2010; Kadarani DK.*et al*.,2015).
Procedure
Preparation of DPPH: 0.1 mM DPPH solution prepared in ethanol.
Standard: Ascorbic acid used as standard for DPPH radical scavenging activity. Different concentration (50, 100, 150 and 200 µg/ml) of ascorbic acid were prepared in ethanol**.**
Control: 1ml of DPPH and 1 ml of ethanol.
EEMZ: Different concentrations (50, 100, 150 and 200 µg/ml) of EEMZ were prepared with ethanol.

For DPPH radical scavenging activity 1 ml of standard/EEMZ and 1ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 30 min. After 30 min, the absorbances of the mixtures were measured by UV spectrophotometer at 517nm, and % inhibition of extract . was calculated against control by using following equation

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DPPH radical sacavenging activity (%) = [{Ao-A1/Ao}] X 100

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Where

Ao is the absorbance of DPPH (Control)

A1 is the abosorbance of DPPH solution in the presence of the Extract (Standard/Sample).

b) *In-vitro* Anti-inflammatory model

1) Heat induced Haemolysis Principle:

The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since RBC (red blood cell) membrane is similar to lysosomal membrane, the study was undertaken to check the stability of RBC membrane.

Procedure:

The reaction mixture (2ml) consisted of 1 ml ethanolic extract of *Manilkara zapota (L.) P. Royen* fruit peel extract of different concentrations (50, 100, 150 and 200 µg/ml) and 1 ml of 10% RBCs suspension, blood was collect from Animal instead of ethanolic extract of *Manilkara zapota (L.) P. Royen* fruit peel extract only saline were add to the control test tube. Diclofenac was used as a standard drug. For heat induced hemolysis all the centrifuge tubes containing reaction mixture was incubated in water bath at 56 ºC for 30 min. At the end of the incubation the tubes was cooled under running tap water. The reaction mixture were centrifuge at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm the experiment was performed in triplicates for all the test samples. The percentage inhibition of Hemolysis was calculated as follows: (Sakat *et al*.,2010; R. Sarveswaran *et al*., 2017;kumari C.S *et al*.,(2015).

$$\textbf{Percentage inhibition} = \frac{\text{(Absorbance of control – Absorbance of sample) X100}}{\text{(Absorbance of control)}}$$

2) Inhibition of Albumin Denaturation assay

Principle:

The denaturation of proteins as one of the cause as inflammation is well documented. Production of auto-antigens in certain rheumatic diseases may be due to *in-vivo* denatuartion of protein. A no. of Anti-inflammatory drugs are known to inhibit the denaturation of proteins. (Akalankadey RP *et al*.,2008).

Procedure:

The reaction mixture(5ml) was consist of 2 ml of test ethanolic extract at different concentration (100, 200, 300, 400 µg/ml) and 0.2 ml 1% aquaeous solution of egg albumin fraction, pH6.4) of the reaction mixture was adjusted using small amount of 2.8 ml of phosphate bufferrd saline and mixture was mixed. The sample were incubated at 37^{o}Cfor 20 min. and then heated to 51^{o}C for 20 min, after cooling the samples the turbidity was measured at 560nm by using UV- visible spectrophotometer . similar volume of double distilled water was used as a control. Diclofenac sodium used as a standard.. The % inhibition of protein denaturation was calculated as follows: (Gunathilake K. D.P.P.*et al*.,2018; Ghumre S.V *et al*.,2017; kumari C.S *et al*.,(2015). Following formula :

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$$\textbf{Percentage inhibition} = \frac{\text{(Absorbance of control – Absorbance of sample) X100}}{\text{(Absorbance of control)}}$$

***In-vivo* study**

a) Anti-inflammatory activity

Evaluation of anti-inflammatory activity was carried out by using Carrageenan(CARR) - induced paw edema. **Carrageenan (CARR)- induced paw edema (**Meshram *et al.*, 2016**).**

Principle:

This model based on the principle of release of various inflammatory mediators by carrageenan. Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin.

The second phase of edema is due to the release of prostaglandins, protease and lysosome. The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through third hour up to five hours.

Procedure:

Wistar rats were weighted and randomly divided into five groups of six animals in each. Group I (control) received normal saline (10 mL/kg p.o.). Group II (Standard) received 20 mg/kg, p.o. of diclofenac sodium. (reference or standard) Group III, IV and V received 100, 200 and 400 mg/kg of EEMZ respectively. Paw edema was induced by (0.1ml) of 1% w/v suspension of carragenan in normal saline wasl injected into sub-plantar region of right hind paw of each rat; left hind paw of animal considered as control. The paw volume was measured at 0 min and after 30, 60, 120 and 180 min of carragenan administration by using plethysmometer means increse in paw volume note down.

. % inhibition of paw edema in drug treated group was campared with the control group **.** % Inhibition was calculated according to formula .

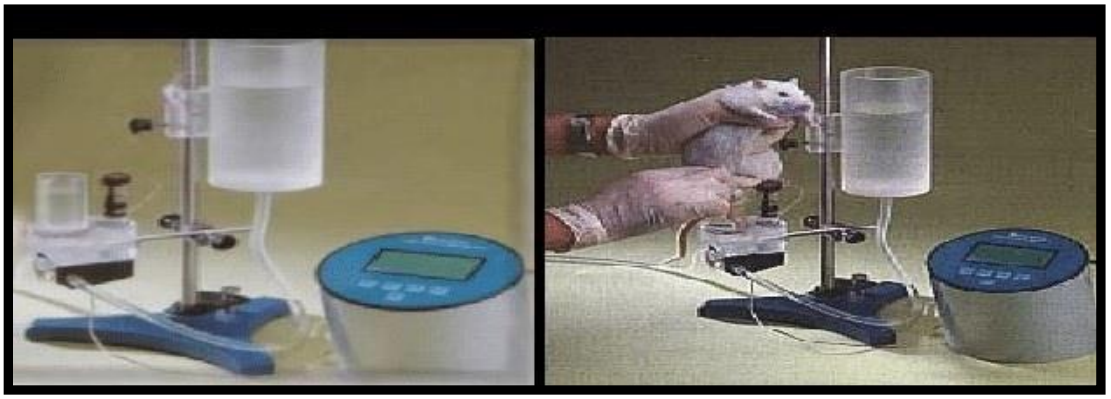

Fig: 6.5 Orchid Scientific Plethysmogram

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$$\% \text{ inhibition} = \frac{(v_t - v_o)\ \text{control} - (v_t - v_o)\ \text{treated}}{(v_t - v_o)\ \text{control}} \times 100$$

V_o= means paw volume at 0 hours (before carragenan injection) V_t= means paw volume at a particular time interval

Table 6.4 Treatment groups for carrageenan induced paw edema of EEMZ

Group	Status	Treatment (N=6 animal in each group)	Parameter
I.	Control	Normal Saline solution (10 ml/kg p.o)	paw volume measure at 0, 30, 60, 120 and 180 min by using Plethysmometer
II.	Standard	Carrageenan 0.1 ml + 20 mg/kg, p.o. diclofenac sodium	
III.	Test	Carrageenan 0.1 ml + 100 mg/kg, p.o. EEMZ	
IV.	Test	Carrageenan 0.1 ml + 200 mg/kg, p.o. EEMZ	
V.	Test	Carrageenan 0.1 ml + 400 mg/kg, p.o. EEMZ	

*EEMZ: Ethanolic extract of *Manilkara zapota Royen* fruit peel

Statistical Analysis :
The data obtained statistically analysed using ANOVA followed by Dunnett's test to detect any significant diffence among different means ,with leval of significance set at $p<0.05$. the result will expressed as mean +- S.E.M.

***In-vivo* Analgesic activity**
In-vivo Analgesic activity of *Manilkara zapota (L.) P. Royen* fruit peel extract extract was carried out by using tail flick test.
a)Tail flick test Procedure:
Swiss albino mice were weighted and divided into five groups with six animals in each group. Group I (control) received normal saline (10 ml/kg p.o.). Group II (Standard) received 20 mg/kg ,p.o. of diclofenac sodium.(standard or reference) Groupand V received 100, 200 and 400 mg/kg. p.o. of EEM respectively. (Sook-Ha-Fan *et al.*, 2014).

About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained At 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia.Maximum Possible Analgesia (MPA) was calculated as follow

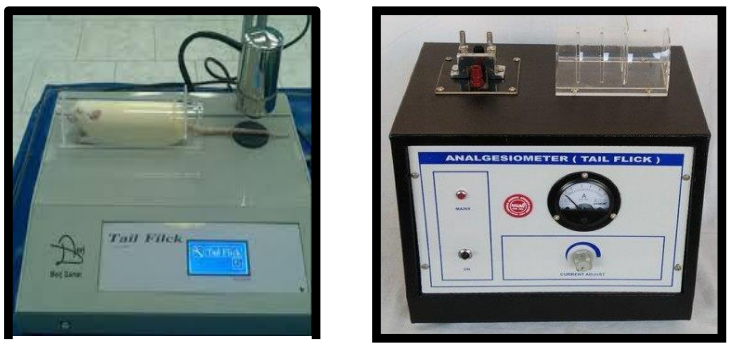


Fig : 6.6 Tail flick analgesiometer

$$\text{MPA} = \frac{\text{Reaction time for treatments} - \text{reaction time for saline} \times 100}{15\ \text{sec} - \text{reaction time for saline}}$$

Table 6.5 Treatment groups for tail flick method of EEMZ

Group	Status	Treatment (N=6 animal in each Group)	Parameter Evaluate
1	Control	Saline solution (10 ml/kg p.o)	The reaction time was recorded before (0min) and at 15 ,30, 45,60 Min after the administration of the treatments
II	Standard	20 mg/kg, p.o. diclofenac sodium	
II1	Test	100 mg/kg, p.o. EEMZ	
IV	Test	200 mg/kg, p.o. EEMZ	
V	Test	400/kg, p.o. EEMZ	

* EEMZ Ethanolic extract of *Manilkara zapota Royen fruit* peel.

7. OBSERVATIONS & RESULT

Calculation:

200gram extract powder required for 1L(1000ml) of solvent

$$(\%)\ \text{Yield} = \frac{\text{Wt. of Extract (6.9 gm)}}{\text{Wt. of powdered drug (200gm)}} \times 100$$

The % yield of *Manilkara zapota* fruit peel was found to be ……**3.45 % w/w.**

Preliminary Phytochemical Studies

Preliminary Phytochemical Evaluation

Table No. 7.1: Preliminary phytochemical evaluations of ethanolic extract of

Manilkara zapota (EEMZ) fruit peel.

Sr. No	Chemical test	Observation
1	**Carbohydrate** 1. Molish test 2. Benedict test	 + +

2	**Alkaloids** 1. Dragendoff's test 2. Mayer's test 3. Wagner's test	 + + +
3	**Glycoside** 1. Borntrager's test 2. Killer Killani test	 – –
4	**Flavonoid** 1. Shinoda test 2. Alkaline reagent test	 + +
5	**Tannins and phenol** 1. Fecl3 test 2. Lead acetate test	 + +
6	**Amino acids** 1. Million's test 2. Ninhydrine test	 + +

***(+):Present, (-):Absent**

Pharmacological Studies

***In vitro* Studies 7.3.1Antioxidant activiy**
(2,2-Diphenyl 1,1--picryl-hydrazyl)radical scavenging (DPPH) Assay

In-vitro Anti-oxidant activity of *Manilkara zapota Royen* fruit peel using DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity.
Several concentrations (50,100 150 and 200 µg/ml) of the ethanolic extract of *Manilara zapota Royen* fruit peel were tested for their antioxidant activity in DPPH (2, 2-dipheny 1, 1- picrylhydrazyl) radical scavenging activity. It was observed that free radicals were scavenged by the Ethanolic extract of *Manilkara zapota Royen* fruit peel in a concentration dependent manner. The Ethanolic extract showed (65%, 68%, 78%, 81%) scavenging activities at 50,
100, 150 and 200 µg/ml concentration respectively. while standard ascorbic acid at 50, 100
,150, 200 µg g/ml concentration showed 71%, 79%, 84%, 89% scavenging activity respectively (Table no: 7.2).

Table: 7.2: DPPH scavenging Activity of EEMZ-Ethanolic extract of *manilkara zapota Royen* fruit peel.

Concentration (µg/mL)	**DPPH radical scavenging activity in %**			
	EEGA		**Ascorbic acid**	
	Absorbance	**% Scavengng activity**	**Absorbance**	**% Scavenging activity**
50	0.22+-0.07	65%	0.185±0.01	71%
100	0.20±0.01	68%	0.130±0.05	79%

150	0.14±0.01	78%	0.099±0.06	84%
200	0.12±0.004	81%	0.069±0.08	89%

Each values represent the mean ± SEM. (N=3) EEMZ- Ethanolic extract of *Manilkara zapota Royen* fruit peels.

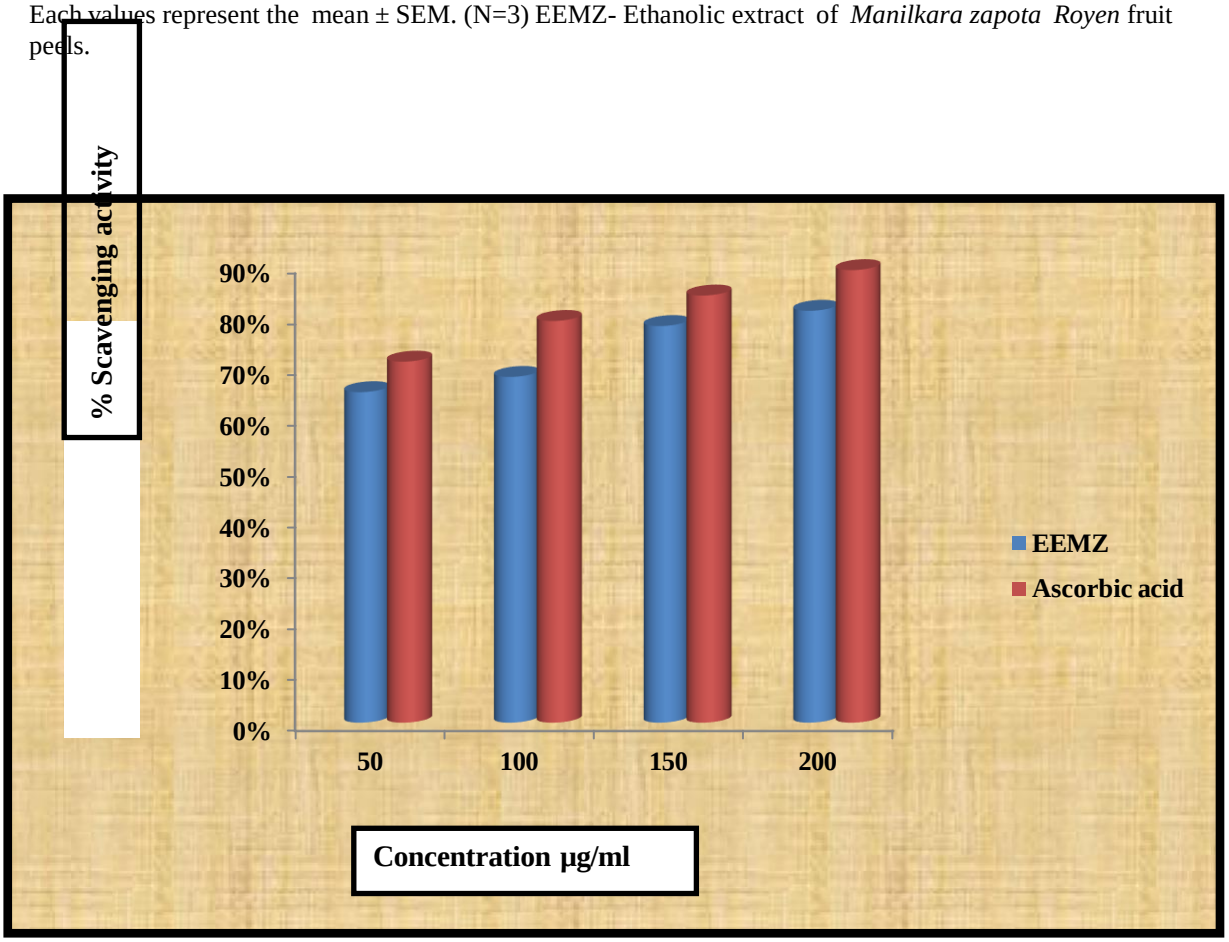


Fig: 7.1: Effect of EEMZ on DPPH radical scavenging activity as % inhibition.

7.3.2 *In-vitro* anti-inflammatory activity

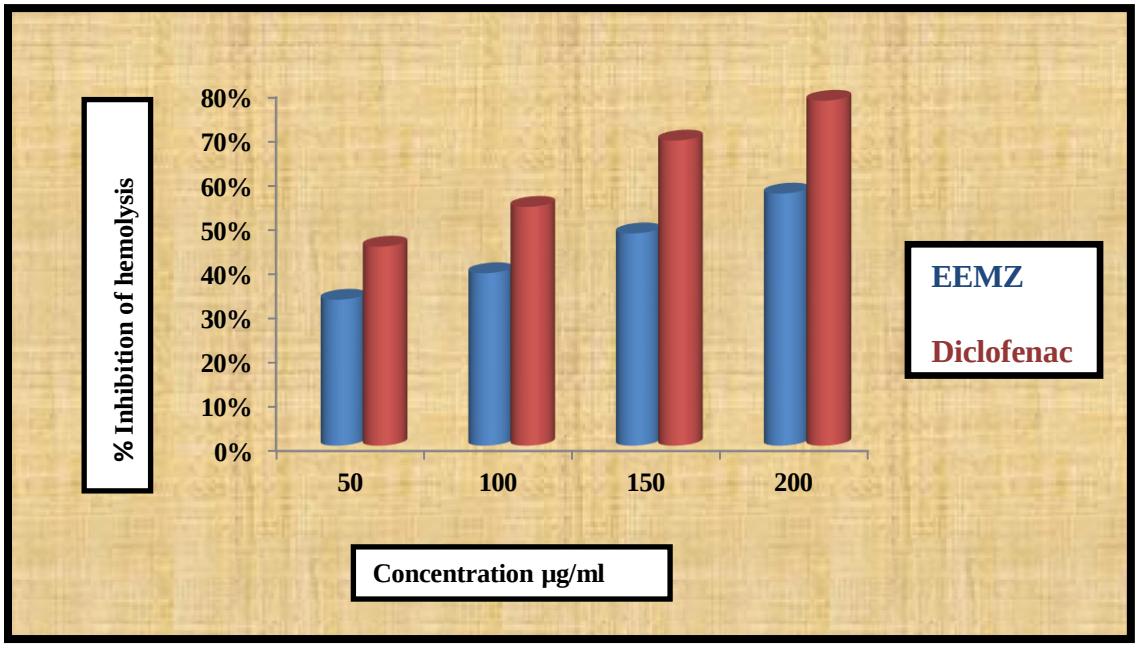
Heat induced hemolysis activity

In-vitro Anti-inflammatory assay of *Manilkara zapota Royen* fruit peel extract was performed using heat induced haemolysis.
The concentrations (50, 100, 150 and 200 µg/mL) of the ethanolic extract of EEMZ *Royen* fruit peel extract were tested for their *in-vitro* anti-inflammatory activity using heat induced hemolysis. It was observed that percent inhibition of ethanolic extract shows 33%, 39%, 48%, 57% at concentration 50, 100, 150 and 200 µg/mL respectively whereas diclofenac sodium as a
standard drug demonstrated inhibition 45%, 54%, 69% and 78% at concentration 50, 100, 150 and 200 µg/mL respectively. (Table no. 7.3).

Table No: 7.3. Heat Induced Haemolysis activity of EEMZ – Ethanolic extract of *manilkara zapota Royen* fruit peel.

Concentration (µg/mL)	Heat Induced Hemolysis			
	EEMZ		Diclofenac	
	Absorbance	% Inhibition	Absorbance	% Inhibition
50	0.22±0.01	33%	0.18±0.008	45%
100	0.20±0.06	39%	0.15±0.01	54%
150	0.17±0.03	48%	0.10±0.01	69%
200	0.14±0.09	57%	0.07±0.02	78%

Each values represent the mean ± SEM. (N=3) EEMZ- Ethanolic extract of *manilkara zapota Royen* fruit peels.



a) Inhibition of albumin denaturation

In-vitro Anti-inflammatory assay of *Manilkara zapota Royen* fruit peel extract was performed using Inhibition of albumin denaturation.
The concentrations (100, 200, 300 and 400 µg/mL) of the ethanolic extract of *Manilkara zapota Royen* fruit peel extract were tested for their *in-vitro* anti-inflammatory activity using Inhibition of albumin denaturation. It was observed that percent inhibition of ethanolic extract shows 63%, 66%, 80%, 85% at concentration 100, 200, 300 and 400 µg/mL respectively whereas Diclofenac sodium
demonstrated inhibition 67%, 80%, 86% and 90% at concentration 100, 200, 300and 400 µg/mL respectively. (Table no. 7.4).

Table No: 7.4. Inhibition of albumin denaturation activity of EEMZ – Ethanolic extract of *manilkara zapota Royen* fruit peel.

Concentration (µg/mL)	**Inhibition of albumin denaturation**			
	EEMZ		**Diclofenac**	
	Absorbance	**% Inhibition**	**Absorbance**	**% Inhibition**
100	0.30+-0.01	63%	0.195+-0.01	67%
200	0.20+-0.01	66%	0.120+-0.02	80%
300	0.12+-0.02	80%	0.081+-0.01	86%
400	0.09+-0.01	85%	0.062+-0.02	90%

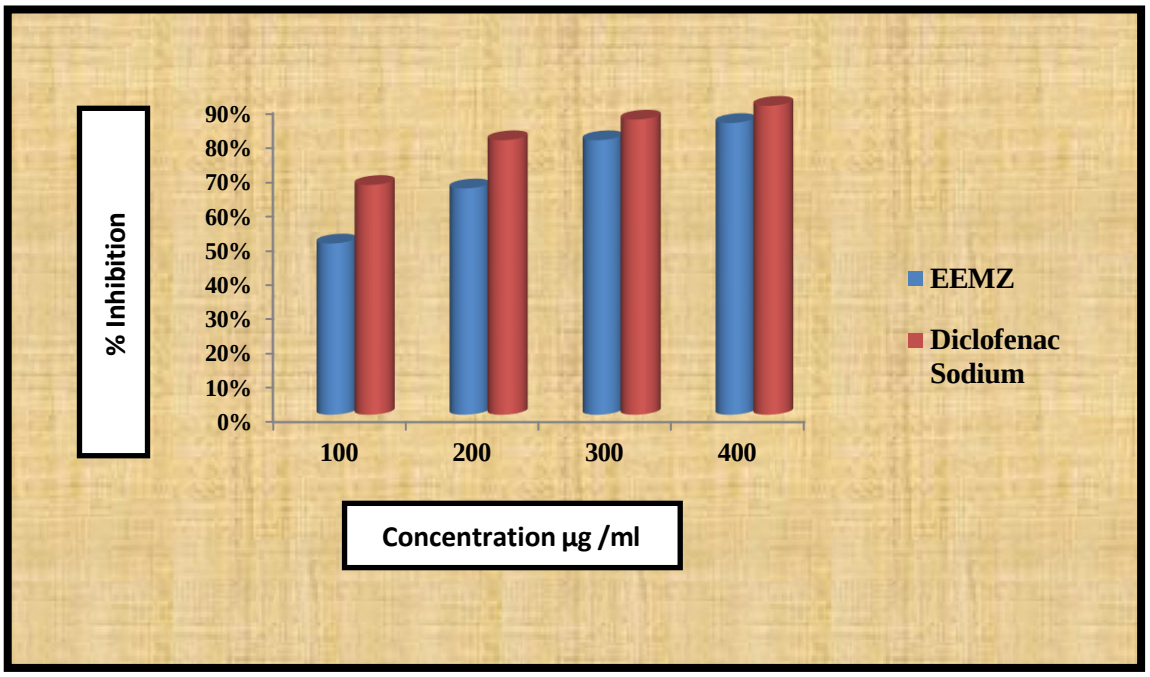


Fig: 7.3 Effect of EEMZ on Inhibition of albumin denaturation assay

Result - *In-vivo* study was not performed due to Covid-19.

8. DISCUSSION

Current active research is focused on herbal medicine in treating inflammation. Herbal medicine obtained from wide array of plant extract are in high usage to cure a wide variety of inflammatory diseases. Side effects associated with NSAIDs drugs made researchers to think about alternative medicines to NSAIDs which is to be natural and free from side effects**.** The significance of natural anti-inflammatory compounds raised interest in pharmacological assessment of variety of plants used in traditional medicine, this intrest resulted in the scientific study of herbal drugs having lesser side effects, thus providing relief to inflammation

Anti-inflammatory, analgesic and antioxidant regarding plants contains a variety of chemical constituents like phenols, coumarins, glycosides, flavonoids, lipids, alkaloids, tannin, saponins that exhibits good Anti - inflammatory and antioxidant activity i.e higher radical scavenging activity .

Certain types of inflammatory injuries are mediated by reactive oxygen species. The most likely sources of these oxidizing agents are the phagocytic leukocytes (e.g., neutrophils, monocytes, macrophages, and eosinophils that invade the tissue .These reactive radicals and oxidants may harm cell and tissues directly via oxidative degradation of cellular components Reactive oxygen species may also initiate and amplify inflammation via the release of several mediators involved in the inflammatory response.

Certain chronic health problems such as asthma, diabetes, hypertension, cancer, kidney and liver failure and certain inflammation, showed involvement of free radicals induced damage. Antioxidants prevent free radical induced tissue damage by stabilizing the unstable free radical by donating electron or may reduce its formation or enhance their decomposition. Free radical induced damage can be reduced by use of certain antioxidant agents derived from synthetic or natural source. The rich sources of natural compounds with antioxidants property are present in traditional Indian diet and medicinal plant, vegetables fruits.

The peel of Sapota fruits were removed from the pulp part and peel were shade- dried for about 10-15 days at room temperature. and then crushed by electric grinder to make a course powder. Extraction of *Manilkara zapota Royen* fruit peel was done by using successive extraction process by using 80% Ethanolic solvent at 40 0C for 24 hours.

After extraction they was filtered in whatman No.1 filter paper and and the petris dishes containing the extract were kept in rota evaporator to solvent get evaporate and get solid state. Then they were defatted with petroleum ether for the further removing fats from extract. Then they were used for further study.

The preliminary phytochemical screening is important for identification of responsible bioactive constituents from the extract those which shows better activity. Such as Flavonoids, tannins, carbohydrates, proteins, alkaloids, saponin, phenols, catechol, terpenoids, steroids, in peel extract of *Manilkara zapota(L). P. Royen* fruit by using standard procedure.

Thus in present study, an efforts was made to evaluate the anti-inflammatory and antioxidant potential of Ethanolic extract of *Manilkara zapota (L). Royen* fruit peel due to presence of certain strong occurrence of polyphenolic compounds such as lipid, proteins, alkaloid, phenols. carbohydrates, phenols, flavonoid and tannin responsible for Anti- inflammatory and antioxidant effect .

The *In-vitro* Anti-inflammatory activity of *Manilkara zapota Royen* fruit peel ethanolic extract was demonstrate by using Heat induced hemolysis and Inhibition of albumin denaturation assay. The extract fraction serve as acting possibly as primary oxidant and heat induced hemolysis stabililized the Red blood cells membrane against lysis induced by heat and inhibited the albumin denaturation. The extract was effective in inhibiting the heat induced hemolysis and inhibition of albumin denaturation at different concentration exhibit a significant scavenging activity and that was compared to control and standard diclofenac sodium as a referance drug.

The *In-vitro* antioxidant activity of *Manilkara zapota Royen* fruit peel ethanolic extract was demonstrated by using DDPH radical scavenging assay. DPPH produce a stable free radical, and in present assay the measurement of electron donating ability of EEMZ and that can be analysed by colour change in the reaction mixture. The changes in colour (from deep violet to light yellow) were measured at 517 nm on a UV-visible light spectrophotometer EEMZ exhibited a significant DPPH radical scavenging activity and that was comparable to ascorbic acid.

9. SUMMARY AND CONCLUSION

Inflammation is a major threat to human health and plays an important role in the development of various infectious and non-infectious diseases such as Alzheimer's, heart disease, asthma, rheumatoid arthritis, etc. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever. Clinical treatment of inflammatory diseases is dependent on drugs, which belong either to the non-steroidal or to the steroidal chemical groups. The use of non-steroidal anti- inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions has potent activity, but long term uses of these drugs have various and severe adverse effects on liver, gastrointestinal tract, etc. Hence, new anti-inflammatory and analgesic drugs lacking such effects are being searched for as alternatives to NSAIDs.
Therefore, a systemic approach has to me made to final out of efficacy of plants against inflammation, pain and for oxidation so as to exploid them as Herbal, Antiinflammatory, analgesic, and antioxidative agent. Owing to safety concerns associated with the use of synthetic anti-inflammatory and analgesic agents, generally the people prefer to take natural anti-inflammatory and analgesic treatments from edible materials such as fruits, spices, herbs and vegetables. Therefore, the development and utilization of more effective anti-inflammatory and analgesic agents with fewer side effects from natural origin are desired. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effect. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory and hepatoprotective agents should therefore be viewed as a fruitful and logical research strategy in search for new drugs.
Manilkara zapota (L.)P.Royen fruit belongs to the family (Sapotaceae), plant of tropical and subtropical regions with a long history of medicinal use. The English name of this fruit is Sapodilla. The plant is abundantly present throughout India. Locally (In Maharashtra)it is known as Chikoo plant. All parts of this plant possess valuable medicinal properties like anti-inflammatory, analgesic, anti-oxidant, anti-diuretic anti- tumor, hepatoprotective.

In Conclusion, present study, the prime objective was to select a medicinal plant which can be used as anti-inflammatory and analgesic agent without side effects. On the basis of literature survey, medicinal uses and

availability of the plant, *Manilkara zapota (L.)P.Royen* fruit peel extract were selected for evaluation of anti-inflammatory, analgesic and and antioxidant activity.
Four major families of compounds were present in the areial fruits peel and may play an important role in anti-inflammatory and antioxidant properties.These families are flavonoids, tannin, phenol, alkaloids, carbohydrates.
In conclusion, present study revealed *In-vitro* anti-inflammatory activity of EEMZ was done using Heat induced hemolysis and Inhibition of albumin denaturation assay most widely used for evaluation of anti-inflammatory properties of medicinal plants. EEMZ shows promising activity in *In-vitro* anti-inflammatory assay. *In-vitro* antioxidant activity of EEMZ was done using DDPH radical scavenging assay. From these *In-vitro* anti-inflammatory and antioxidant studies of *Manilkara zapota (L.) P. Royen* fruit peels ethanolic extract showed significant anti-inflammatory and anti-oxidant effect according to concentration manner. These potential anti-inflammatory and antioxidant effects were found to be referable due to presence of certain phytochemical such as tannin, flavonoids, carbohydrates, lipid, alkaloids, saponin may be responsible for the activity.
Our results are encouranging as the source for NSAIDs drug are natural and hence, it may not have side effects like synthetic molecules. Further investigation are required to find active component of the extract and to confirm the mechanism of action. From result of *in-vitro* anti-inflammatory and antioxidant assay, it could be concluded that the ethanolic extract of *Manilkara zapota* has been found to have shown good anti-inflammatory and antioxidant activity. The result of this study indicates that referring to folk literature is a helpful approach to identify plants with bioactive potential.

REFERENCES

[1]. Abdulkhaleq L.A, Assi MA, R. Abdullah, M. Zamri-Saad, Y.H. Taufiq-Yap, M.N.Hezmee. The crucial role of inflammatory mediators in inflammation: *A review. Veterinary world* (2018);**11(5)**:627-635.
[2]. Abheri, DS, Anisur, RM, Ghosh AK. Free Radicals and Their Role in Different Clinical Conditions: An Overview. *International Journal of Pharma Sciences and Research*, (2010);**1(3)**: 185-192.
[3]. Adwas AA, Ibrahim Elsayed AS, Azab E, Quwaydir FA. Oxidative stress and antioxidant mechanism in human body. *Journal of applied biotechnology and bioengineering*,(2019);**6(1):**43-47.
[4]. Ali K, Majlesi M, Mahmoud Rafieian-K. Herbal versus synthetic drugs;beliefs and facts. *Journal of Nephropharmacology*, (2015); **4(1):** 27-30.
[5]. Amlan Ganguly, Zobaer Al Mahamud, In-vivo anti-inflammatory and anti-pyretic activity of Manilkara zapota leaves in albino Wistar rats. Asian *Pacific Journal of Tropical Disease*© (2013);**3(4)**: 301-307.
[6]. Anbudhasan P, Surendraraj S, Satishkumaram S. Natural anitioxidant and its benefits.
[7]. *International journal of food and nutritional sciences*,(2014);**3**:225-32.
[8]. Arora A. Arora K. Textbook of Pathology. *Pulse publication*,(2016);11-833.
[9]. Ashley, NT. Weil ZM, Nelson RJ. Inflammation: Mechanisms, Costs and Natural Variation. *Annual Review of Ecology, Evolution, and Systematics*, (2012);**43(1):**385– 406. http://doi.org/10.1146/annurev-ecolsys-040212-092530.
[10]. Asnakech Alemu, W. Tamiru, T. Nedi, W. Shibeshi. Analgesic and Anti- inflammatory effects of 80% Methanol Extract of Leonotis ocymifolia (Burm.F.) Iwarsson leaves in Rodents Models. *Hindawi Evidence-based Complementary and Alternative Medi*. © (2018);**Vloume2018**:8 pages Article ID 1614793https;//doi.org/10.1155/2018/1614793
[11]. Atta EM, Mohamed NH, Abdelgawad ahmed AM. Antioxidants: An overview on the natural and synthetic types. *Eur. Chem. Bull*.,(2017);**6(8):**365-
[12]. 75.DOi:10.17628/ecb.2017.6.365-375.

[13]. Ayesha Khan. Evaluation of Analgesic and Anti-inflammatory activity of whole plant Extract of Manilkara zapota linn.*World journal of pharmacy and pharmaceutical science research article* (2016);**Volume 5**:881-92 DOI: 10.20959/wjpps20167-7081
[14]. Bagad A, Joseph N, Bhaskaran A, and Agarwal A. Comparative evaluation of anti- inflammatory activity of curcuminoids, turmerones, and aqueous extract of Curcuma longa. Advances in Pharmacological Sciences, (2013); **3**: 7.
[15]. Barbara J.A, Xaveer Van O, Angel F L. Tumour necrosis factor-alpha(TNF-α): The good, the bad and potentially very effective. *Review article Immunology and cell biology Institute of medi. and verterinary science* (1996);**74**:434-443.
[16]. Biswas S, Das R, E. ray Banerjee. Role of free radicals in human inflammatory diseases. *Immunobiolgy S. and regenerative medicine Research laboratory AIMS Biophysics* (2017);**Volume 4(4):**596-614

[17]. Bogani P, Galli C, Villa M. Francesco V. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atherosclerosis* 2007; **190(1)**:181-186.
[18]. Borgeat P, Samuelsson B. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. *Journal of Biological Chemistry* (1979); **254**: 2643-6.
[19]. Boullata I, Nace M. Safety issues with herbal medicine. *Pharmacotherapy*, (2000);
[20]. **20**: 257-269.
[21]. Brown JE, Rice-Evan CA. Luteolin-rich Artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radical Research*, (1998); **29**: 247-255.
[22]. Chanda SV, Nagani KV. Antioxidant capacity of Manilkara zapota L. Leaves Extracts Evaluated by Four in vitro Methods. *Nature and science* (2010);**8(10):**260-
[23]. 266. ISSN:1545-0740).
[24]. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. *et al.* Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget,(*2017);**9(6):**7204-218.
[25]. Colin D. Funk. Prostaglandins and Leukotrines: Advances in Eicosanoid Biology.
[26]. *Science*, (2001);**294**:1871
[27]. Cumpstey A, Feelisch M. Free radicals in inflammation. *Wiley- VCH verlag GmbH and Co. KGaA*. ©(2018);695-726.
[28]. Dahl V, Reader JC. Non-opioid postoperative analgesia. *Acta Anaesthesiologica Scandinavica*, (2000); **44**: 1191-1203.
[29]. David Rosenbloom, Marilyn A. A Review of Non-Steroidal Anti-Inflammatory Drugs Craven Can *Fam Physician*. (1983); **29**: 2121–2124.
[30]. Denko CW, A role of neuropeptides in inflammation. *Biochemistry of Inflammation. London*: ©*Kluwer Academic Pub.* (1992); 177-181.
[31]. Donowitz M, Arachidonic acid metabolites and their role in inflammatory bowel disease. *Gastroenterology*,(1985);**88**:580-87.
[32]. Dr. Parle Milind, Preeti, Chickoo: A wonderful Gift From nature. *Int. J. Res. Ayurveda Pharm.* 2015;**6(4):**544-550 http://dx.doi.org /10.7897/2277-4343.064102.
[33]. Francesca Seta, M. Bachschmid. Cyclooxygenase pathway of the Arachidonate cascade. *Advanced article john Wiley and Sons, Ltd*.© (2012)DOI: 10.1002/9780470015902.0023401.
[34]. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Laboratory investigation; *a journal of technical methods and pathology*, (2003**); 47(5)**: 412-426.
[35]. Gahol M, Bhatt P, Joshi J. Study on yield of plant extract using different solvents and method. *Bulletin of environment, pharmacology and life sciences*.(2018);Vol 7: 65-67.
[36]. Ghurme SV, Swant M, Jadhav VM, Kadam VJ, Sonawne N, Ramaiya M. Cynodon dactylon and acyclovir showing synergistic effect by albumin denaturation and membranr stabilization assay. *Mod.Appro.Drug.Des.*,(2017);**1(2):**1-5.
[37]. Gomathy K, Baskar R, Kumaresan K. Comparison of antioxidant potential in pulp and peel extracts of Manilkara zapota (L.) P. Royen. *African journal of Biotechnology*, (2013);**12(31):**4936–943.
[38]. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. In vitro Anti-inflammatory properties of selected Green leafy vegetables. *Biomedicines*,(2018); **6(7)**: 1-10.
[39]. Habib I, MD, Mazulis A, Roginsky G, Ehrenpreis Eli D. NSAIDs and inflammatory bowel Disease: Pathophysiology and Clinical Associations. *Inflamm Bowel Disease*
[40]. *.ibdjournal.org*. (2014);**20**:2493-2502.
[41]. Halliwell B. free radicals, antioxidant and human disease: curiosity, cause, or consequence. *lancet*, (1994);**Vol 344**:721-724.
[42]. Holt P, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study, *Digestive Diseases and Sciences*, (2005); **50(11):** 2191–93.
[43]. Hossain MH, Jahan F, Howlader islam MS, Dey SK, Hira A, Ahmed A, Sarkar RP. Evaluation of Anti-inflammatory activity and total flavonoids content of Manilkara zapota (Linn) bark. *International Pharm. Phytopharmacol, research* (2012);**2(1)**:35- 39.
[44]. Hossain, Hemayet. Evaluation of anti-inflammatory activity and total flavonoids content of Manilkara zapota (Linn.) Bark. *Inernational Journal Pharm Phytopharmacol Research*, (2012);**2(1)**: 35-39.
[45]. Huwiller A, J. Pfeilschifter. Lipid as target for novel anti-inflammatory therapies.
[46]. *pharmacology and therapeutics* (2009);**124**:96-11
[47]. Jain PK, PS, Evaluation of Analgeisc activity of Manilkara zapota (leaves) Pelagia Research library.

European Journal of Experimental biology,(2011),**1(1):**14-17
[48]. Jaradat N, Hussen F, Ali AA. Preliminary phytochemical screening, quantitative estimation of total flavonoids ,total phenol and antioxidant activity of ephedra alata decne. *J. Mater. Environmental science*, (2015);Vol6 (6):1771-778.
[49]. Jenitha A, Bhuvaneshwari M. In vitro studies on phytochemical analysis and antioxidant activity of manilkara zapota. *International journal of research in pharmacology and pharmacotherapeutics*, (2016);**Vol 5(2):**153-161,issue 2.
[50]. Jignasu P, Pravin H, Sohil H, *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of Cassia species, *Arabian Journal of Chemistry*, (2017); **10**: S1654–S1662.
[51]. John AO. Ojewole, Antinociceptive, anti-inflammatory and antidiabetic effect of Bryophyyum pinnatum leaf aqueous extract. *Journal of ethnopharmacology* (2005);**99**:13- 39
[52]. Johnson J, Sohn D, Inman F, Bjeldanes R, –Lipophilic stinging nettle extracts possess potent anti-inflammatory activity, are not cytotoxic and may be superior to traditional tinctures for treating inflammatory disorders, *Phytomedicine*, (2013); **20(2)**: 143–
[53]. 147.
[54]. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. *Alternative Medicine Review*, (2009); **14**: 141-53.
[55]. Juvekar Anti-inflammatory activity of aqueous extract of barleria cristata leaves. *Journal of Young Pharmacists*, (2009);**1(3):**220-226 http://doi.org/10.4103/0975- 1483.57068
[56]. K.H.S, Peiris, Sapodilla. Fruit crop *research and Development centre Horana, Sri lanka* (2014).https://www.researchgate.net/publication/261707049
[57]. Karle P, Dhawale SC. Manilkara zapota (L.) P.Royen fruit peel: *A phytochemical and pharmacological Review*, School of Pharmacy, S.R.T.M.U. Nanded-431606, Maharashtra, India (2019);**10(1):**11-14.
[58]. Kazemipoor. Safety, Efficacy and Metabolism of Traditional Medicinal Plants in the Management of Obesity: A Review. *International Journal of Chemical Engineering and Applications*, (2012);**3(4)**: 288–292.
[59]. Khalvat A. Comparing the effects of ginger (Zingiber officinale) extract and ibuprofen on patients with osteoarthritis. *Archives of Iranian Medicine*, (2005); **8(4)**: 267–271.
[60]. Khanna HD, Karki K, pande D, Negi R, Khanna RS. Inflammation, Free radical damage, Oxidative stress and cancer. *Interdisciplinary Journal of Microinflammation* 2014; **1(1):**1-5.
[61]. Kumar V, Abbas Ak, Aster JC. Robbins Textbook of Basic Pathology. *Philadelphia, Pennsylvania:Elsevier*,©(2018);**10th edi**.:57-96.
[62]. Kumari CS, Hussain Babusevam M. Invitro- anti-inflammatory and anti-arthitic property of Rhizopora mucronata leaves. *International journal of pharma science and research.* (2015);**Vol 6**:482-485.
[63]. Kumari K, Biswas H, Analgesic Activity of Quisqualis indica, *The Pharmaceutical and Chemical journal*,(2017);**491**:1-8
[64]. Lobo V, Patil A, Pathak A, Chandra N. Free radical, antioxidant and functional foods:Impact on human health. *Pharmacognosy reviews*, (2010);**4**: 118-26.
[65]. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *British Journal of Pharmacology*, (2006); **147**: S232-S240
[66]. Mahluji SA, Ostadrahimi M, Mobasseri VE, Attari, Payahoo L. –Anti- inflammatory effects of Zingiber officinale in type 2 diabetic patients,‖ *Advanced Pharmaceutical Bulletin*, (2013); **3(2):** 273–276.
[67]. Maiti B, Nagori BP, Singh R, Kumar P, Upadhyay N. Recent trends in herbal drugs: A review. *International journal of drug research and technology*, (2011);**Vol1(1):**17- 25.
[68]. Mandal S, Yadav S, Kumar R Nema. Antioxidant : A review. *Journal of chemical and pharmaceutical research*,(2009);**1(1)**:102-104.
[69]. Manna A, Abalaka ME. Preliminary screening of the various extracts of Physalis angulata (L.) for antimicrobial activities. *Spectrum Journal*, (2000); **7**: 119-25.
[70]. Marmitt DJ, Bitencourt S, Amanda Do Couto ES, Marcia IG, C. Rempel. Medicinal plants of renisus with analgesic activity. *Journal of critical reviews*, (2016);**3**:1-4.
[71]. Medhitov R. Inflammation 2010: new advantures of an Flame cell. (2010);**140**:771- 76.
[72]. Mehnaz B, Ahmed B, Manilkara zapota (L.)P.Royen (sapodilla):A Review. *International Journal Of Advance Research, Ideas And Innovation In Technology*,©(2017);**Volume 3**: 1364-371,Issue 6.

[73]. Mehta JP. In-vitro antioxidant and in-vivo anti-inflammatory activities of aerial parts of Cassia species. *Arabian Journal of Chemistry*, (2017);**10**:S1654–S1662. http://doi.org/10.1016/j.arabjc.2013.06.010
[74]. Meshram G.G, Anil K, Waseem R, C.D Tripathy, R.A. Khan. Evaluation of anti- inflammatory activity of the aqueous and ethanolic extract of the leaves of Albizzia lebbeck in rats. *Journal of Traditional and Complementory Medicine*,(2015);1-4 http://dx.doi.org/10.1016/ j.jtcme.2014.11.038.
[75]. Mohamad Ali H, Ahmed EI-M, Maha Aboul-E, Abdallaa E. Evaluation of analgesic activity of Papaver Libanoticum Extract in mice: Involvement of opioids receptors. *Hindawi Evidence-Based Complementory and alternative medicine*,(2017);**Volume2017**:1-13, https://doi.org/10.1155/2017/8935085.
[76]. Mohan H. Textbook of Pathology. *New Delhi: Jaypee publication(P) LTD*,(2010);**6th**
[77]. **edi.**:130-171.
[78]. Naclerio RM, Baroody FM, Togias AG. The role of leukotrienes in allergic rhinitis: a review. *Am Rev Respir Dis* (1991); **143**: S91-95.
[79]. Nakhata N. Thromboxane A2: Physiology/ pathophysiology, cellular signal transduction and pharmacology. *pharmacology and Therapeutics*, (2008);**118**:18-
[80]. 35.doi:10.1016/j.pharmthera.2008.01.001.
[81]. Nandini S. Roy A, Kumar J. Plants with analgesic property –A review article. *Oral medicine and radiology*,(2018);**10**: 3847-850,issue 5.
[82]. Navghare VV, Dhawale SC. In vitro antioxidant, hypoglycemic and oral glucose tolerance test of banana peels. *Alexandria Journal of medicine*, (2016);1-7.
[83]. Nile, S.H., Se Won Park.Optimized Methods for In Vitro and In Vivo Anti- Inflammatory Assays and Its Applications in Herbal and Synthetic Drug Analysis. *Mini- Reviews in Medi. Chem*. (2013);**13**:95–100 DOI: 10.2174/138955713804484712.source:pubMed.
[84]. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Manilkara zapota.
[85]. *Agroforestry database :a tree referances and selection guide version*, (2009);**4**:1-5.
[86]. Padmavathi D. A study on nutritional and health importane of sapotas. *International journal of food science and nutrition*, (2018);**Vol 3**:184-87.
[87]. Pahwa R, Goyal A, Bansal P, Jialal I. chronic inflammation. *Statpearls Publishing,*
[88]. (2020).
[89]. Pingale R, Kumar dash G. Pharmacognostic Evaluation of manilkara zapota (L.) P. royen root. *International journal of pharmacognosy and phytochemical research*,(2015);**7(3)**:405-08.
[90]. Pradeepkumar MR, Joshi SD, Kulkarni VH, Savant C. Phytochemical screening and evaluation of analgesic and anti-inflammatory activities of phaseolus vulgaris linn., seeds in rodents. *Journal of Applied pharmaceutical sciences*, (2015);**5(06):**066-69.
[91]. Punchard NA, Whelan CJ, Adcock I. *The journal of inflammation*. (2004);**1(1):**1-4.
[92]. Rafieian-Kopaei M. Medicinal plants and the human needs. *Journal of Herbal Medicine Pharmacology* (2012); **1(1):** 1-2.
[93]. Randall H, Randall F, Dobbs C, Hutton H. –Randomized controlled trial of nettle sting for treatment of base-of-thumb pain. *Journal of the Royal Society of Medicine*, (2000); **93(6):** 305–309.
[94]. Ricciotti E, G.A FitzGerald. Prostaglandin and Inflammation. *Arterioscler Thromb Vasc biol*, (2011);**31**:986-1000 DOI:10.1161/ATVBAHA.110.207449
[95]. Roger GB. The enzymes of the human Eicosanoids pathway. *Research and reports in medical sciences*,(2018);**Vol 2**:1-28.
[96]. Roschek B, Fink C, McMichael M. Nettle extract (Urtica dioica) affects key receptors and enzymes associated with allergic rhinitis, *Phytotherapy Research*, (2009); **23(7):** 920–26.
[97]. S.D. Sheorey, Madhuri A. Hinge. Analgesic and Anti-inflammatory Plants :An
[98]. updated review, *International journal of pharmaceuticals Sciences Review and Research* January (2012);**Volume 12**;Article-022 ,25/1/15
[99]. Saha A, Ajmed M. The analgesic and anti-inflammatory activity of the extract of Albizia lebbeck in animal model. *Pakistan Journal of pharma Science.* (2009); **22(1)**:4-7
[100]. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. *International Journal of Pharma and Pharmacological Sciences*, (2010); **2(1)**:146-155.
[101]. Samuelsson B. An Elucidation of the arachidonic acid cascade discovery of prostaglandin, thromboxane and leukotrienes.(1987);**1**:2-9.

[102]. Sarveswaran R, Jayasuriya WJABN, Suresh TS In vitro assays to investigate the anti- inflammatory activity of herbal extract: review. *World journal of pharmaceutical research*, (2017);**Volume 6**:131-41,issue 17.DOI:10.20959/wjpr201717-10058.
[103]. Schaffer M, M.D. Thomas B, MS; Horst D.B, M.D. Thomas K. Hunt , MD. Neuropeptides. *Dept. of surgery* (2020);**Volume 133**:11071116.
[104]. Schuppan D, Jia D, Brinkhaus B, Hahn G. Herbal products for liver diseases: a therapeutic challenge for the new millennium. *Hepatology*, (1999); **30**: 1099-1104.
[105]. Schuppan D, Stikel F. Herbal medicine in the treatment of liver diseases: *Digestive and liver diseases*, (2007); **39**: 293-304.
[106]. Serhan CN, Brain SD, Buckley CD, Gilroy DW.Resolution of inflammation: state of the art, definition and terms. *The FASB journal*, (2007);**21**:325-32.
[107]. Shaikh R.U, Mahesh M, pund, Rajesh N. Gacche. Evaluation of anti-inflammatory activity of selected medicinal plants used in traditional medication system in vitro as well as in vivo. *Journal of Traditional and Complementory Medicine*(2015);1-7 http://dx.doi.org/10.1016/j.jtcme.2015.07.001
[108]. Shukla S, Mehta A, Mehta P, Vyas SP, Bajpai VK. Studies on antiinflammatory, antipyretic and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. *Food and Chemical Toxicology*, (2009); **48**: 61-64.
[109]. Simmons DL, Botting RM. Cyclooxygenase isoenzymes: the biology of prostaglandin synthesis and inhibition. *Pharmacological Review*,(2004);**Vol 56:**387- 437.
[110]. Singh M, Kumar V, Singh I, Gauttam V, Kalia NA. Anti-inflammatory activity of aqueous extract of mirabilis jalapa linn. leaves. *Pharmacognosy research*,(2010);**Volume 2**:364-67.
[111]. Sook-Ha fan, Noraisah Akbar A. Evaluation of Analgesic Activity of the Methanol extract from the Galls of Quercus infectoria (Olivier) in Rats. *Evidence-Based Complementory and Alternative medicine. (*2014); **Volume 2014**:1-6pages http://dx.doi.org/10.1155/2014/976764
[112]. Spector WG, Willoughby DA. The inflammatory response. *Bacteriological Reviews*, (1963); **27**: 117-54.
[113]. Sreejith G. Anti-allergic, anti-inflammatory and anti-lipidperoxidant effects of Cassia occidentalis Linn. *Indian Journal of Biology*. (2010) ; **48(5)**: 494-8.
[114]. Suralkar AA, Rodge KN. Evaluation of Anti-inflammatory and Analgesic activity of Tamarindus indica seeds, *Internatonal journal of Pharmaceutical Sciences and Drug Research* (2012);**4(3):**213-217
[115]. Tiwari S. Plants: a rich source of herbal medicine. *Journal of Natural Products*, (2008); **1**: 27-35.
[116]. Tracy RP. The five cardinal signs of inflammation: calor, Dolor, Rubor, Tumorand penuria. *Journal of Gerontology a Biological science medicinal science*, (2006);**Vol 61**:1051-52.
[117]. Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action.
[118]. Inflammation research supplement 2, (1998):S78-S87.
[119]. Vodovotz Y, Csete M, Bartels J, Chang S, An G. Translational systems biology of inflammation. *PLoS Computational Biology*, (2008);**4(4)**:1-6, doi:10.1371/journal. pcbi.1000014
[120]. Wang H, Cao G, Prior RL, Total antioxidant capacity of fruits. *Journal of Agriculture and Food Chemistry*, (2008); **44**: 701–5.
[121]. White M. Washington MD, DC. Mediators of inflammation and inflammatory process. *The Journal of Allergy and Clinical Immunology*, (1999); **103**: S378-81.
[122]. Winter CA, Risley, Nuss EA, GW. Carragenan -induced edema in the hind paw of rat as an assay for anti-inflammatory activity. *Proc. Soc. Exp. Biol. Med*.,(1962);**111**: 544-47.
[123]. Wong Rebecca SY. Role of NSAIDs in Cancer prevention and cancer promotion.
[124]. *Hindawi Advances in pharmacological sciences*, (2019); **Vol 2019:**1-10.