# Potentiation of the Anti-inflammatory Effects of NSAIDs, Steroids and Statins Using Antioxidants

A project submitted

by

Tanvir Ahmed

ID: 13146021

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This work is dedicated to my parents and my respected project supervisor Fabliha Ahmed Chowdhury (Lecturer, Department of Pharmacy, BRAC University) from whom I received the most support.

# Certification statement

This is to certify that, the project titled 'Potentiation of the Anti-inflammatory Effects of NSAIDs, Steroids and Statins Using Antioxidants' submitted for the completion of the precondition for the degree of Bachelor of Pharmacy to the Department of Pharmacy, BRAC University, contains my personal work under the supervision of Fabliha Ahmed Chowdhury, Lecturer, Department of Pharmacy, BRAC University. Proper acknowledgement goes to those from whom I got the ideas.

Signed,

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Counter signed by the supervisor

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# Acknowledgement

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# Abstract

Inflammation is the normal defensive mechanism in our body, but in some cases it may be responsible for causing varieties of diseases. In those kinds of situations, it becomes a major concern for our normal health condition. For this instance, there are several anti-inflammatory drugs present for the treatment of inflammatory diseases. However, the conventional anti-inflammatory drugs give long term side effects. Thus, it is better to use them in a low dose for a shorter duration of time. We have used antioxidant in our project work in order to find out the enhancement of the therapeutic effect of the anti-inflammatory drugs like Diclofenac (NSAID), Prednisolone (steroid) and Atorvastatin (statin). Inflammation was induced in two ways using carrageenan and formalin. Carrageenan produced local inflammation and formalin induced neuropsychiatric effects. The inhibitions of such responses have been measured using a drug alone and in combination with antioxidant. The result of this study has shown that in case of carrageenan mediated inflammation, the combination of 5mg/kg Diclofenac and 200mg/kg Vitamin C has given the highest inhibition of 74.19%. On the other hand, the poorest performance was shown by the combination of 5mg/kg Prednisolone and 100mg/kg Vitamin C giving an inhibition of 32.57%. The 100mg/kg & 200 mg/kg Vitamin C have given respectively 18.98% and 20.95% inhibition alone. In case of formalin mediated inflammation group, the combinations of 5mg/kg Diclofenac and 200mg/kg Vitamin C have given 97.25% inhibition. On contrary, the combinations of 8mg/kg Atorvastatin and 100 mg/kg Vitamin C have given 50.55% inhibition only. Here, 100mg/kg & 200 mg/kg Vitamin C have given respectively 94.51% & 97.53% inhibition alone. According to the results, the combination of 5mg/kg Diclofenac and 200mg/kg Vitamin C have given the most inhibition in both local and neuropsychiatric inflammation induced by respectively carrageenan and formalin. So, this study actually recommends a new combination of drug therapy which will work against both local and neuropsychiatric effect caused by inflammation.

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## Abbreviations

| | |
|---|---|
| COPD | Chronic obstructive pulmonary disease |
| LT | Leukoterine |
| TNF-alpha | Tumornecrosis factor |
| IL-8 | Interleukin-8 |
| IL-6 | Interleukin-6 |
| WBC | White blood cell |
| IL-1 | Interleukin-1 |
| Cox | Cyclooxygenase |
| Cox-1 | Cyclooxygenase-1 |
| Cox-2 | Cyclooxygenase-2 |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| HOCl | Hypochlorous acid |
| DNA | Deoxyribonucleic acid |
| RNA | Ribonucleic acid |
| HMG-CoA | 3-hydrooxy-3methylglutaryl-coenzyme |
| LDL | Low density lipoprotin |
| HDL | High density lipoprotin |
| NSAIDs | Nonsteroidal anti-inflammatory drugs |
| PONV | Post-operative nausea and vomiting |
| LPS | Lipopolysaccharide |
| FDA | Food and drug administration |
| ROS | Reactive oxygen species |
| RNS | Reactive nitrogen species |
| SOD | Superoxide dismutase |
| GSHPx | Glutathione peroxidase |
| CAT | Catalase |
| ICDDR,B | International Centre for Diarrhoeal Disease Research, Bangladesh |
| NaCl | Sodium Chloride |
| CRP | C-reactive proteins |

# Chapter One: Introduction

Inflammation is body's normal, defensive response to tissue damage which is caused by physical injury, toxic chemicals or other harmful microorganisms. It is the process to neutralize or eliminate invading organisms and unwanted substances and also to repair the tissue injury in the body. After accomplishing the healing process, the inflammatory mechanism generally become less intense (Whalen et al, 2015).

## 1.1 Pathophysiology of Inflammation

Inflammation is a response which is intended to remove the initial debris of cell injury, like necrotic cells and other tissues coming from different sources. It causes motion of the events which eventually fix and repair the sites of tissue trauma. It reacts in a complex way, causing vascular changes, transfer and excitation of leukocytes and other systemic reactions (Roche et al, 1996).

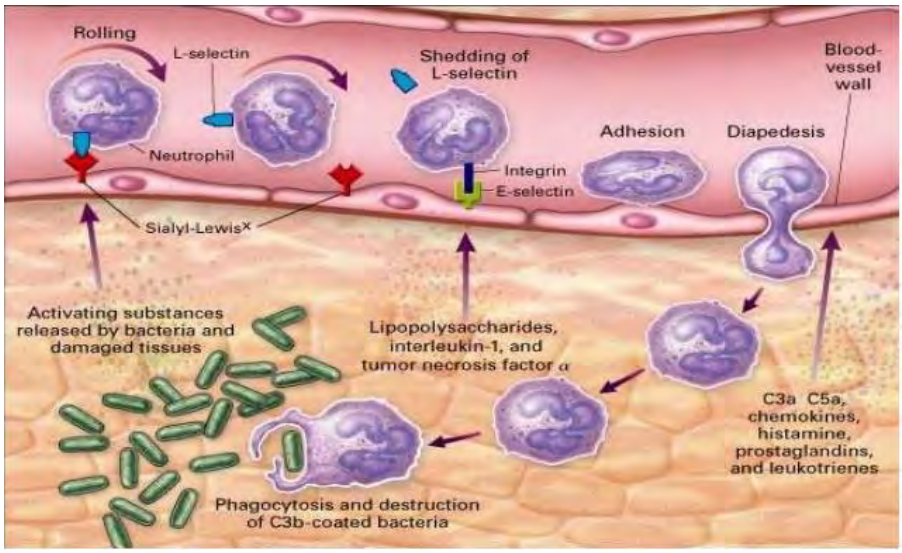


**Figure 1.1: The Pathophysiology of Inflammation** (Weatherspoon, 2017)

The activation of WBC results in activation of T lymphocytes, a subdivision of lymphocyte which is a subtype of WBC that plays a significant role in cell-mediated immunity, activates and stimulates the monocytes and macrophages. Those cells discharge pro-inflammatory cytokines, which include tumour necrosis factor (TNF)-α and interleukin (IL)-1, inside the synovial pit (Craig & Stitzel, 2004).

The arrival of cytokines at that point causes increase cell penetration into the endothelium because of arrival of histamines, kinins, and vasodilatory prostaglandins. It

also increases generation of C-responsive protein by hepatocytes (a marker for aggravation), enhances production and arrival of proteolytic chemicals by chondrocytes (cells that look after ligament), prompting corruption of ligament and joint space narrowing, expanding osteoclast action, bringing about central bone disintegrations and bone demineralization around joints and foundational appearances in specific organs, for example, the heart (Whalen et al, 2015).

### 1.2 Stages of Inflammation

The underlying inflammation stage comprises of three sub stages: acute, sub-acute, and chronic (proliferative). The three stages of inflammation show various kind of response in the body which lasts for few days to several months.

#### 1.2.1 Acute Inflammation

The acute stage ordinarily keeps going 1– 3 days and is portrayed by the five exemplary clinical signs: heat, redness, swelling, pain, and loss of capacity. The stimuli for the acute inflammations are bacterial, viral, parasitic and microbial toxins, trauma (blunt and penetrating) (Cannon & Hough, 2007).

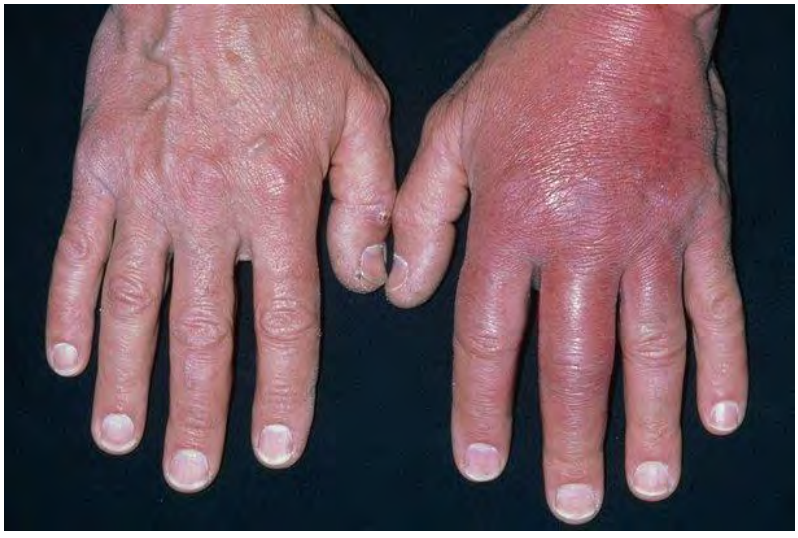

**Figure 1.2: Symptoms of Acute Inflammation** (Nimse & Pal, 2015)

It also involves the physical and the chemical agents, thermal injury, for example, burns or frostbite, irradiation and some other environmental chemicals, tissue necrosis; foreign bodies (dirt) and immune reactions which are also called hypersensitivity reactions. According to Lewis's experiment, the sign of the acute inflammation include- changes in

the skin colour by triple response in a few seconds because of local vasodilation. They are swelling, redness and heat generation in that region. The swelling is referred to formation of edema. Similarly, redness is due to vasodilation and finally heat is produced due to excess of blood coming to the cell that is injured (Cannon & Hough, 2007).

### 1.2.2 Sub-acute Inflammation.

The sub-acute stage may last from 3– 4 days to 1 month and compares to a cleaning stage required before the repair stage. In the event that the subacute stage isn't settled within 1 month, at that point inflammation is said to be noticeably endless and can keep going for a while. Tissue can decline and chronic inflammation may prompt enhancing. Then again, after the subacute inflammatory stage, tissue can restore and be fortified amid the redesigning stage (Craig & Stitzel, 2004).

The subacute stage is defined by development of phagocytic cells to the site of damage. In light of adhesion, molecules discharged from enacted endothelial cells, leukocytes, platelets, and erythrocytes in harmed vessels wind up noticeably sticky and hold fast to the endothelial cell surfaces. Polymorph nuclear leukocytes, for example, neutrophils are the principal cells to penetrate the site of damage (Tak et al, 2015). Basophils and eosinophils are more pervasive in hypersensitive responses or parasitic contaminations. As inflammation proceeds, macrophages prevail, evacuating harmed cells or tissue. The sub-acute period of inflammation might be trailed by the time of tissue repair (Molina et al, 1985). Blood clusters are evacuated by fibrinolysis, and injured tissues are recovered or supplanted with fibroblasts, collagen and endothelial cells. (Tak et al, 2015).

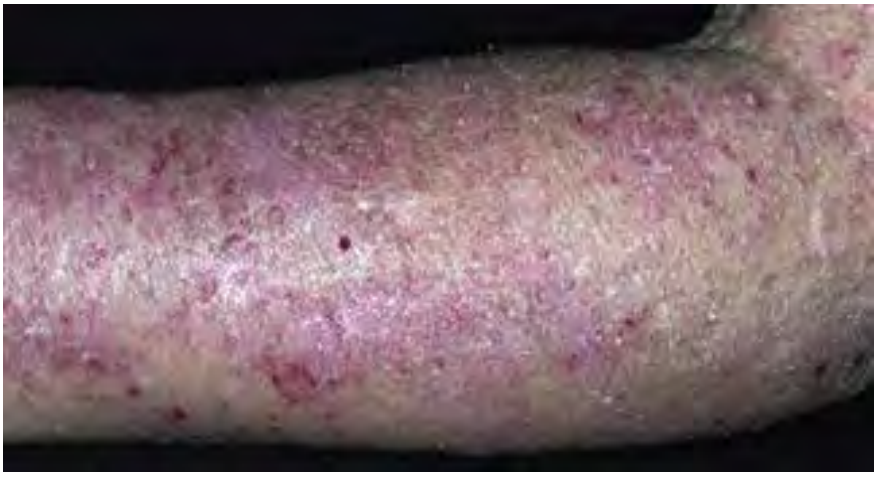

**Figure 1.3: Sub-acute Inflammation** (Nimse & Pal, 2015)

### 1.2.3 Chronic Inflammation

The chronic inflammation can define the long-lasting inflammation and can persist for several months to years. It results from failure to remove what was causing the acute inflammation. An autoimmune reaction to a self-antigen of the immune system attacks the normal healthy cells, where it is mistaking it for the harmful pathogens. It occurs due to exposure to a minimum level of an irritant, for instance a chemical agent, for a long period of time (Kruif et al, 2007).

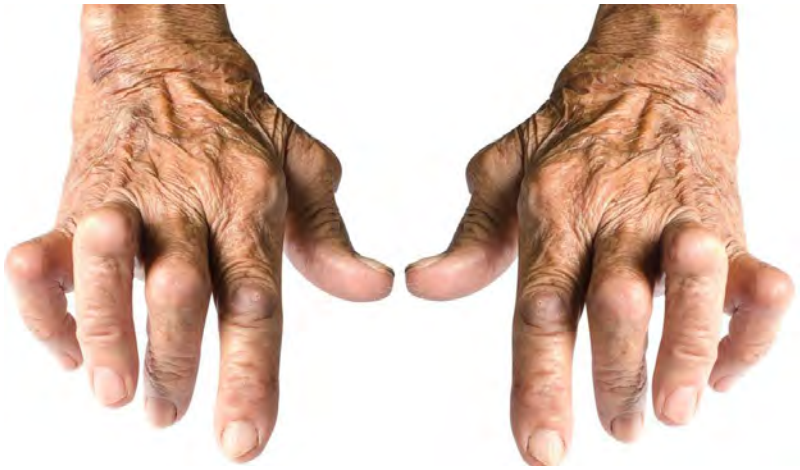

**Figure 1.4: Result of Chronic Inflammation** (Tak et al, 2015)

Examples of the diseases and the conditions which include chronic inflammation is asthma, chronic peptic ulcer, rheumatoid arthritis, tuberculosis, chronic periodontitis, ulcerative colitis, chronic sinusitis and chronic active hepatitis etc. As we know damaged tissue could not repair without inflammation, the chronic inflammation can ultimately cause varieties of diseases and such conditions including cancers, rheumatoid arthritis, periodontitis and hay fever (Tak et al, 2015). In any case, not all chronic inflammatory illnesses comprise of the hazard of malignancy and some of them, for example, psoriasis, may even lessen it. But, at the end it causes huge pain at the inflammatory site (Kruif et al, 2007).

# Chapter Two: Mechanism of Inflammation

The mechanism of inflammation involves two pathways. One is the WBC pathway and another is the Oxidative Pathway.

## 2.1 Mechanism of WBC (White Blood Cell) Pathway

White blood cells (WBCs), also termed leukocytes, are the major cells of the immune system in our body which are involved in defencing or protecting our body against the infectious diseases and the foreign substances. All WBC are generated and derived from multiple cells in bone marrow which is known as hematopoietic stem cells. The leukocytes are observed throughout our body, which include the blood and the lymphatic system (Cekici et al, 2013).

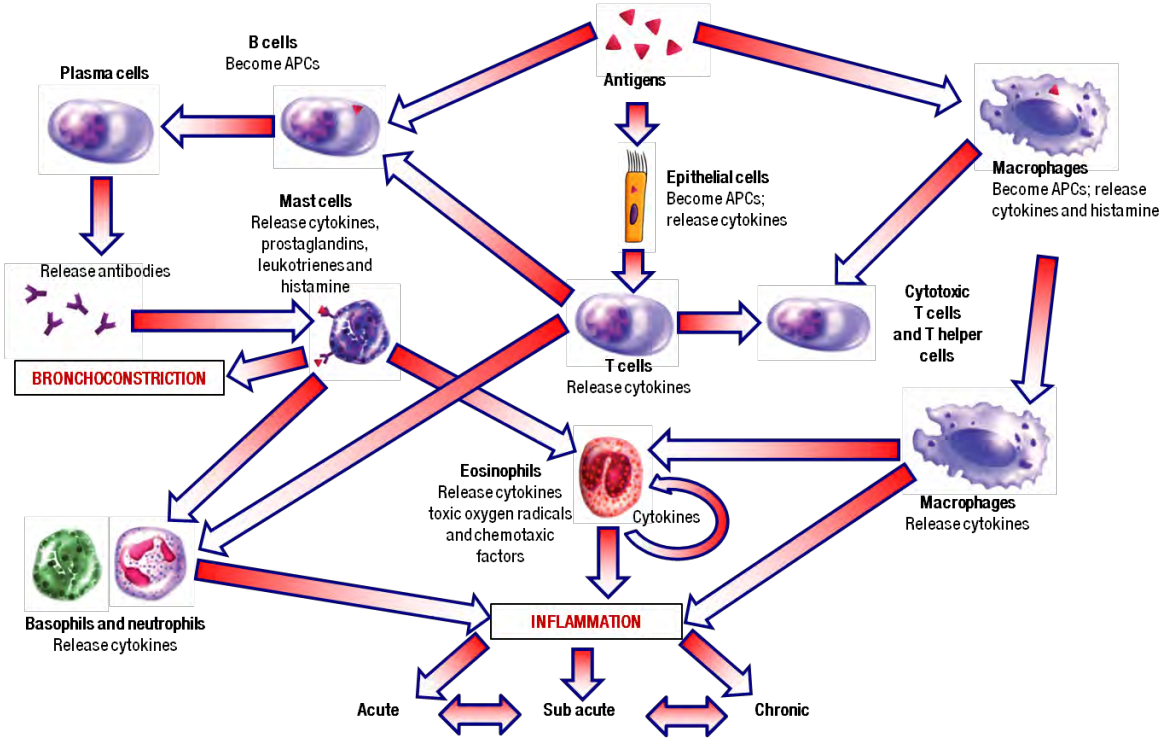


**Figure 2.1: The WBC Pathway of Inflammation** (Zeka et al, 2006)

When the leukocyte penetrates connective tissue, it should be able to find and enter to the site of injury. This is done by chemotaxis that depends on the ability of leukocytes to sense a chemical signal through its cell body and then migrate to the directions of the site where the chemical is concentrated. The agents for chemotactic signalling include- neutrophils (the products of complement system), monocytes (the bacterial exudants, neutrophil cationic substances). Lymphokines discharged from the sensitized

lymphocytes, other complement substances (C567 complex, C3a and C5a) (Zeka et al, 2006).

Prostaglandins are one of them which contribute to inflammatory process. It is one of the potent mediators that increase in blood flow, chemotaxis (the chemical signals which stimulate WBC) and reduce the functional activities of tissues and other organs. Like inflammatory process prostaglandin is continually produced against the noxious response (Ricciotti & Fitzgerald, 2011).

Similarly, tissue injury takes place the enzyme which is called phospholipase A2 is released that converts the cell membrane substance phospholipids into arachidonic acid. The arachidonic acis is the substrate of two major enzymes called Cyclooxygenase or Cox and 5-lipoxygenase. The Cox enzymes are mainly of two types Cox-1 & Cox-2. The Cox-1 isoform is disclosed constantly throughout the body. Its major function is production of thromboxane and prostaglandin which is responsible for pain, inflammation and fever in the body (Williams et al, 1999).

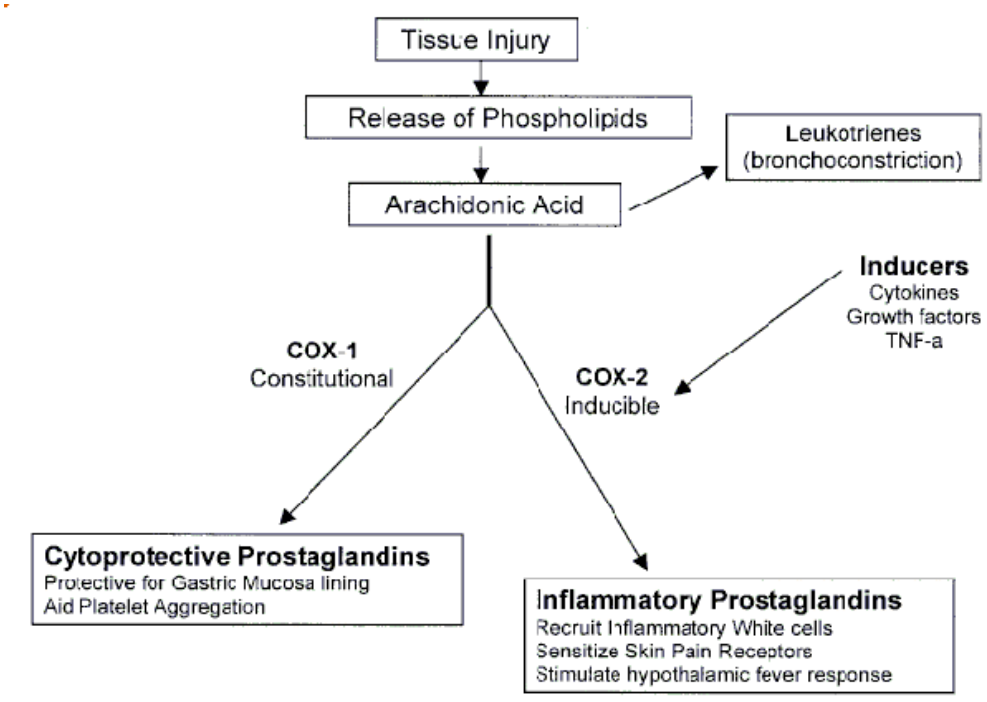


**Figure 2.2: The Cox Pathway to Induce Inflammation** (Wang & Dubois, 2009)

On the other side, Cox-2 isoform is not disclosed constantly throughout the body rather than in most tissues. Unlike Cox-1, Cox-2 acts mainly at the site of inflammation. Cox-2 derived prostaglandins mediate inflammation, pain and fever (Williams et al, 1999).

## 2.2 Mechanism of Oxidative Pathway

Another way to induce inflammation is the generation of free radical and the process is called oxidation. When an infection occurred, the neutrophils come to fight against the infection. There are two mechanisms that involve killing the microbes, one is oxygen dependent and another is independent. In oxygen dependent mechanism oxygen is needed must. Generally it is started with the oxidative burst, where an enzyme called NADPH (Nicotinamide adenine dinucleotide phosphate) oxidase that takes the oxygen molecule and converts it into superoxide ($O_2$-) which is further converts into hypochlorous acid (HOCl). But the main reaction to produce the free radicals is the conversion of oxygen into superoxide (Rosenson & Stafforini, 2012).

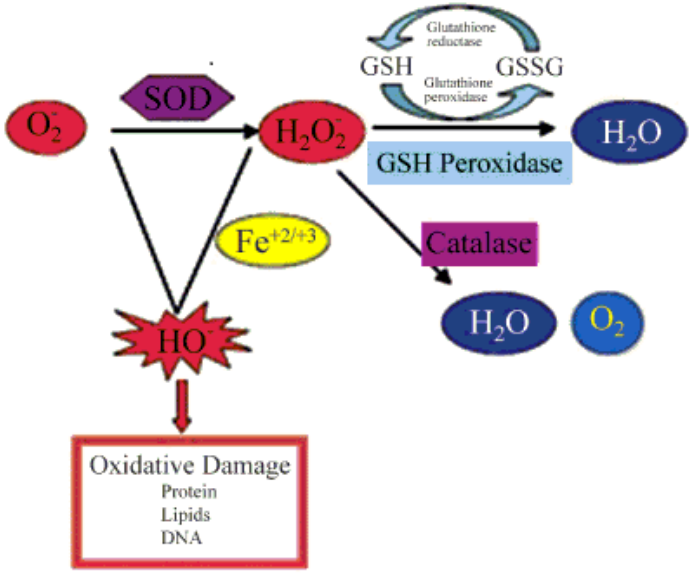


**Figure 2.3: Formation of Superoxide and Subsequent Cell Destruction** (Lee & Wolfgang, 2015)

These free radicals induce inflammation by destruction of cell membrane. The free radicals take an electron from the cell membrane constituent lipid because it has an unpaired electron which needs an electron from the cell membrane lipid. For this reason the cell membrane lipid leaves with an unpaired electron and this is repeated continuously leading to the formation of a chain reaction which results in breakdown of cell membrane causing inflammation. These free radicals can also oxidize the proteins and also the DNA inside the cell and so on (Rosenson & Stafforini, 2012).

## Chapter Three: Reasons behind the Occurrence of Inflammation

There are several sources which are responsible for inflammation. It may cause from tissue injury to pathogenic attack and also from physical and mental stress. There are also other reasons behind the occurrence of inflammation, for example dietary problem, sleeping problem, consumption of alcohol, smoking cigarette and many more. The sources of inflammation are described below.

### 3.1 Injury

Injury is the main reason behind the occurrence of inflammation. There are many types of injuries including small cut to massive wound, bruise, trauma, tear, rent, lesion, sore and so on. Without the inflammation process, the wound healing process cannot complete. In some cases inflammation cause the destruction of cell (Kruif et al, 2007).

### 3.2 Pathogens

Pathogens are the major source of inflammation. 70% of our immune cells reside in our intestines, so our intestinal bacteria can affect our immune system in many ways. The bacteria which are inside our gastrointestinal tract can suppress inflammation or initiate inflammation depending on their nature. For example, *Helicobacter pylori* are bacteria in the stomach which contribute to inflammation and subsequently developing ulcers. This is the reason because of using probiotics try to influence the intestinal inflammatory response. The scientists do not understand this mechanism yet, but they're finding that the environmental and the dietary changes affect the way our micro biomes regulate inflammation. Studies discovered that definite microbes seem to be related to producing rheumatoid arthritis and Crohn's disease, which are inflammatory diseases (klein, 2015). Inflammation initiated by microbiota is thought to become worse other diseases as well as HIV. Several examples of microbes that cause inflammation followed by chronic disease: *Mycoplasma pulmonis* causes chronic lung disease, *Ureaplasma urealyticum* causes pneumonia and *Chlamydophila pneumonia* causes chronic asthma etc. (Cassell, 1998).

### 3.3 Stress

There are two types of stress; chronic and acute stress. The stress hormone is cortisol that has a major role in regulating the cellular inflammatory response. Chronic stress also

moreover likes to enhance the production of WBCs, which increases the possibility of inflammation-related diseases. So, both the acute and chronic stress is a major concern for inflammation (Williams et al, 1999).

On the other hand, the stress also can be divided by two types- physical stress and mental stress. The physical stress includes low energy, upset stomach, headache, constipation, diarrhoea, nausea, muscle pain and tense, etc. The mental stress includes anxiety, depression, major depressive disorder, fear, insomnia and many more. These stressful conditions may lead to inflammation (Sali et al, 2012).

### 3.4 Obesity

Excess body weight can result in inflammatory reactions within the fat cells. With increasing the age, some cells in the fat tissue of body become aged and they cause inflammation. High diet sugar, processed food, refined carbohydrates, carbonated water, soft drinks and junk food etc. are very much responsible for obesity which result in inflammation. Moreover, obesity is a major cause of the inflammation (klein, 2015).

### 3.5 Smoking

Every puff of a cigarette smoke irritates our lungs, leading to a few degree of inflammation which can be worsen the existing lung complication like COPD (Chronic obstructive pulmonary disease) or allergies. On the other hand, some experts think that the chronic trauma and inflammation inside lungs from smoking is the reason behind the beginning of cell mutation that causes lung cancer. It is shown that smoking enhancing various markers of inflammation, including a high WBCs count and excess levels of C-reactive protein, a substance which is produced by liver. Fortunately, after quitting this habit, many inflammatory markers have been dropped dramatically (Cannon & Hough, 2007).

### 3.6 Alcohol

Alcohol is broken down inside our body. It produces toxic by-products which promote inflammation. One problematic result of heavy alcoholism is steatosis also known as fatty liver. The accumulation of fat inside the liver results in chronic liver inflammation which in turn leads to hepatitis or cirrhosis. So, excessive alcoholism is another source of inflammation (klein, 2015).

### 3.7 Contraceptive Pills

Pre-menopausal women who are taking the oral contraceptives are likely to be more prone to low class of inflammation. Recent study found that 30% of pre-menopausal women taking pills had very high levels of the inflammation indicator C-reactive protein, while only 7% of pre-menopausal women not using the pill suffered from the same (Lee & Wolfgang, 2015).

# Chapter Four: Anti-inflammatory drugs

We have chosen four types of anti-inflammatory drugs for this study which will be described below.

## 4.1 NSAIDs

### 4.1.1 Indications

NSAIDs are generally used for the treatment of diseases like acute or chronic disease where pain and inflammation are observed. It is used for osteoarthritis, rheumatoid arthritis, low back pain, mild-to-excess pain because to inflammation and tissue trauma, inflammatory arthropathies, tennis elbow, migraine, headache, acute gout, dysmenorrhoea (menstrual pain), postoperative pain, metastatic bone pain, muscle stiffness and trauma because of Parkinson's disease, pyrexia (fever), renal Colic and ileus etc. They are also administered to neonate infants who are suffer in ductus arteriosusis and macular edema etc. Aspirin is the only NSAID which is able to irreversibly inhibit COX-1 and also (Silverstein et al, 2000).

### 4.1.2 Mechanism of Action

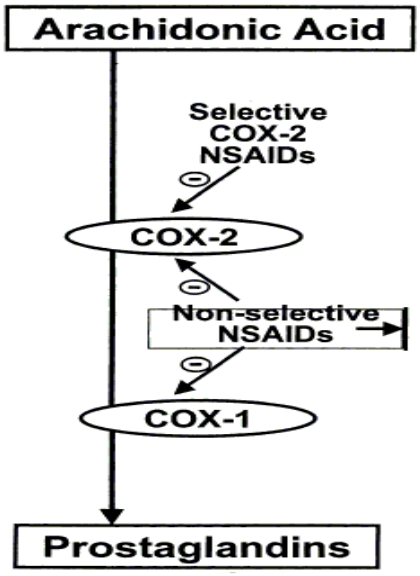


**Figure 4.1: Mechanism Actions of NSAIDs** (Grivennikov et al, 2010)

The conventional NSAIDs are non-particular inhibitors of cyclooxygenase (COX) and restrain similarly both isoforms, the COX-1 and the COX-2. NSAIDs are arranged by their selectivity in restraining the two isoforms of COX. During the tissue injury arachidonic acid is formed. The arachidonic acid is then stimulating the formation of prostaglandins through the cox pathway which responsible for pain associated inflammation and fever. The NSAIDs are blocked the Cox pathway thus inhibiting the formation of prostaglandins.

### 4.1.3 Side-Effects

The worldwide use of NSAIDs has observed that the side effects of these agents are increasing day by day. Use of NSAIDs enhances the risk of gastrointestinal (GI) problems. NSAIDs are usually used for the pain management after accomplishment of surgery. On the other hand, there is information that NSAIDs increased threat of kidney diseases. Their use in gastrointestinal operation remains questionable. About 10–20% of NSAID patients suffer from dyspepsia. High doses of NSAIDs were related with serious upper gastrointestinal side-effects which include bleeding. NSAIDs, like all drugs, may interact with other medications. Other side-effects of NSAIDs include nausea, vomiting, diarrhoea etc (Wang & Dubois, 2009).

### 4.1.4 Contraindications

There are certain factors and conditions that can intensify a patient's risk of NSAID induced gastro-intestinal bleeding. The major factors include history of ulcer disease, advanced age, poor health status, treatment with certain drugs, the duration of NSAID therapy, smoking, and heavy alcohol use. These agents have certain effects on kidney. So, patients with renal impairment, heart failure, hypertension, and edema must use these agents with appropriate caution. If a patient has the history of hypersensitivity reaction to Salicylates or any other similar NSAIDs, then these drugs are strongly contradicted for them. Also the asthmatic patients are more prone to suffer from these reactions, thus asthmatic patients should also take these agents with proper caution (Xian & Zhou, 2009).

In most of the cases, NSAIDs are associated with some common drug interactions. By the concomitant use of corticosteroids (long term), other NSAIDs, bisphosphonates, or anticoagulants NSAID induced gastro-intestinal toxicity may increase. If anti-coagulants

like Warfarin are co-administered with certain NSAIDs, they can compete for protein binding sites that may result into GI bleeding. Similar situation may occur if myelosuppressive antineoplastic drugs that cause thrombocytopenia are coprescribed. NSAIDs may lower the drug clearance rate of Methotrexate, which can cause serious haematological and GI toxicity. This condition is likely to happen when high dose of Methotrexate is administered for treating psoriasis or cancer. NSAIDs have certain mechanism to lower the synthesis of prostaglandin in kidney. As a result they may sometimes increase the nephrotoxicity of these agents - aminoglycosides, amphotericin B, cidofovir, cisplatin, cyclosporine, foscarnet, ganciclovir, pentamidine, and vancomycin. These agents can also lower the excretion rate of Lithium like drugs. Because NSAIDs often decrease their activity, some drugs which are used as antihypertensive, such as diuretics should be taken with caution. Hepato-toxicity and increased hepatic enzymatic activity can also occur (Xian & Zhou, 2009).

## 4.2 Steroid

### 4.2.1 Indications

The steroid also established anti-inflammatory drug. But long time use of steroid drug has long time effects which is very harmful for our body. Steroids are also known as the ultimate drug, which has wide range of activity (Kruif et al, 2007).

The steroids are one of the most universally used types of drugs and they have huge role in the therapy of inflammatory, pulmonary, dermatological and oncological diseases which has been well known. There is an enhancing of application of steroid therapy at the time of perioperative period for several purposes. Some of the known indications are Perioperative replacement therapy, Anti-inflammatory uses and hyper-reactive airway, Analgesia adjunct, Post-operative nausea and vomiting (PONV), Day care surgery, Septic shock, Anaphylaxis and other indications like – cerebral edema, spinal cord trauma and several surgical causes. Steroids have many effects on various tissues that are dose dependent (Sali et al, 2012).

### 4.2.2 Mechanism of Action

Steroids totally revise cellular and also humoral immune reactions. Different mechanisms are involved in the inhibition of inflammation by use of steroids. They supress the production of different inflammatory elements which are very critical in

resulting and propagating the inflammatory mediators like interleukins, cytokines, and chemotactic agents. For this instance there is a declined secretion of vasoactive and chemo-attractive mediators and also diminished the secretion of lipolytic and other proteolytic enzymes, declined extravasation of leucocytes to the site of injury. The ultimate effect of these reactions on several immune cells, consequence in an abolished inflammatory response. Anti-inflammatory action is usually observed with the higher doses of steroid (Sali et al, 2012).

### 4.2.3 Side-Effects

There are many side-effects in relation to the usage of steroids. The side-effects include severe acne, oily skin and loss of hair, liver disease, heart disease, kidney disease, altered mood, irritability, depression, increased aggression, alterations in cholesterol and other types of blood lipids, high blood pressure, shrinking of testicals, gynecomastia, azoospermia, menstrual irregularities in women, excess facial or body hair, infertility, deeper voice in women, height in teens, Risk of viral and other bacterial infections because of unsterile injections (Kruif et al, 2007).

### 4.2.4 Contraindications

There are huge contradictions concerning the use of steroids. The contradictions include peptic ulcers, psychoses, infections, hypertension and diabetes. For an instance, in the iatrogenic adrenal insufficiency, prednisolone and other such glucocorticoids, should be reduced gradually and treatment should be discontinued if the therapy continue more than one week. Insulin dependent diabetics may also need increased in insulin doses if any patient is treated with steroids (Kruif et al, 2007).

## 4.3 Statin

### 4.3.1 Indications

Generally the statin is an anti-cholesterol agent which lowering the cholesterol level in our body. It is widely used in the patients associated with risk of cardiovascular disease. Although the mechanism action of statin as an anti-inflammatory agent is not established yet. But recently researchers have found that the statin may have anti-inflammatory action. Statins are generally used to lower the “bad” LDL levels from 21% to 63% and to increase “good” HDL levels 4% to 16%. Studies have shown that, in case of some

people, statins decrease the risk associated with heart attack, stroke and death from heart disease by a ratio of 25% to 35% (Water, 2010).

Statins have also other effects except lipid-lowering action in the defence of atherosclerosis. It is improved endothelial function, lowering the oxidative stress of body tissues, decreased inflammation, it is also maintained plaques to prohibit further block in arterioles and constriction and finally prevention of blood clots which may form plaques and cross to other heart and brain spaces (Water, 2010).

### 4.3.2 Mechanism of Action

Statins aggressively hinder the protein 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the primary submitted venture of sterol amalgamation, and lower plasma cholesterol levels. They have been broadly explored in clinical trials as cholesterol lowering specialists to diminish bleakness and mortality from coronary vein sickness. Late clinical trials have demonstrated that statins lessen the danger of cardiovascular occasions indeed, even without a noteworthy diminishing in blood cholesterol levels, proposing that the advantages of statin treatment may likewise be credited to their activity on non-lipid factors engaged with endothelial brokenness, nitric oxide bioavailability, inflammation– fibro proliferation and plaque dependability, essential highlights of atherosclerosis (Nezic et al, 2009).

Moreover, high-measurements simvastatin shows intense vasoprotective properties amid endotoxaemia that might be helpful for patients with intense foundational aggravation and related vascular hyporeactivity. In addition, atorvastatin was an intense and compelling endothelium-defensive specialist that lessened leucocyte-endothelial cell cooperations in vivo. In vitro investigations of mitigating activities of statins on cell occasions have been directed to clarify the impacts past the lipid-bringing down ones. Restraint of HMG-CoA reductase movement in human monocytes treated with lipopolysaccharide (LPS) lessened the creation of IL-8, IL-6 and MCP-1, chemotactic cytokines that are communicated in human atherosclerotic injuries and in charge of leucocyte enrollment at the disease site. In this examination, we meant to test potential calming impacts of atorvastatin in intense neighbourhood inflammation and furthermore to contrast and that of NSAIDs and Steroids, a notable anti-inflammatory agent (Sparrow et al, 2000).

### 4.3.3 Side-Effects

Most common complain of taking statins is muscle pain. The pain may lead to a mild discomfort or it may be enough harmful to make daily activities hard. Rarely, the statins may cause life-threatening muscle injury that is called rhabdomyolysis. Very rarely, statin could cause enhance enzymes which signal to liver inflammation. It is possible to increase blood glucose level by intake of statins that may cause advancing type 2 diabetes. The FDA aware that people taking statins may developed loss of memory and confusion (Moosmann & Cohl, 2004).

### 4.3.4 Contraindications

The statin drugs associate with pregnancy category X, which means fetal malformation can be occurred. In case of pregnant and breastfeeding women, statins are not allowed. Statins are contraindicated in patients who have active liver disorder. In this case the liver couldn't work properly and is unable to excrete medications effectively. This may result in accumulation of drug in the body which causes harmful effects. It may also lead to liver disorder to progress quicker causing fully liver failure. Statins may lead to increase liver enzymes. Increased liver enzymes cause loss of liver function. Individuals may also face an allergic reaction to statin characterize by itching, swelling in hand and face, trouble in breathing and chest tightness (Water, 2010).

## 4.4 Anti-oxidant

### 4.4.1 Indications:

Generally anti-oxidant is a common dietary supplement in our daily food routine. The anti-oxidant include Vitamin C, Alpha Lipoic acid, Glutathione, etc.Vitamin C helps to treat scurvy. Most of the people believe that it helps to treat common cold. Scientists have found that the ascorbic acid has the anti-cancer property and it also helps to cure the cardiovascular disease and other types of chronic disease, such as rheumatoid arthritis (Nimse & Pal, 2015).

### 4.4.2 Mechanism of action:

Antioxidants are the molecules which inhibit the free radical reactions and supress cellular damage. Although antioxidant defenses vary from species to species, in the presence of

antioxidants defence is unique. Antioxidants remain both in enzymatic and non-enzymatic types in the intracellular and extracellular situation (Nimse & Pal, 2015).

In case of regular biochemical reactions, exposure presence of higher levels of dietary xenobiotics lead to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are liable for the oxidative stress in variety of pathophysiological situation. Cellular substances of our body in oxidative stress stages causing different disease conditions. The oxidative stress may significantly be neutralized by intensify cellular defenses in the state of antioxidants. Many substances may act as *in vivo* antioxidants by enhancing the levels of internal antioxidant defenses. In case of expression of genes encoding different enzymes for example superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT) enhance the level of internal antioxidants (Nimse & Pal, 2015).

### 4.4.3 Side-effects

Constipation, diarrhea and upset stomach can be observed. These side effects are mostly temporary and will disappear as the body adjusts to this agent. Iron may turn stool color in black, an effect which is not harmful. Most people using this agent do not face any serious side effects. Serious allergic reaction to this drug is very rare (Nimse & Pal, 2015).

### 4.4.4 Contraindications

Anti-oxidants have very minor problem in case of intake of the drug. But some patients are observed with allergic reaction by taking anti-oxidant (Nimse & Pal, 2015).

## Chapter Five: Rationale

The aim of the study was to identify whether new drug regimen for the inflammatory diseases can be established or not (Abbas et al, 2014). The rationale behind the study is given below:

1. In this study, the anti-oxidant was used to observe its anti-inflammatory effect alone and also with the combination of NSAID, Steroid and Statin. As we know that the anti-oxidants neutralize the free radicals, this helps to reduce the tissue damage.
2. As we know that the common anti-inflammatory drug NSAIDs and Steroid have huge side effects, so there is a necessity of new treatment regimen is essential to treat inflammatory diseases. The NSAIDs such as Aspirin, Diclofenac, Ibuprofen, Naproxen and Celecoxib etc. have a lots of side effects include gastric irritation, ulceration, heartburn, headaches, dizziness, ringing in the air, increase blood pressure, kidney dysfunction and leg swelling etc (Wheeler, 2005). Similarly, long term use of Steroidal drugs (Betamethasone, Prednisone, Dexamethasone, Cortisone, Hydrocortisone, Methylprednisolone, Prednisolone) include complications like osteoporosis, high blood sugar, high blood pressure, stomach ulcer, emotional side effects, such as irritability and clouding of the lens in eye, excitability, cataracts. So, we wanted to observe the potentiation effect using anti-oxidants, as that would reduce the dose and duration of use of these drugs (Abbas et al, 2014).
3. This study was performed to judge which combination would work best as treatment regimen. So we can easily judge if the statin and anti-oxidant give better anti-inflammatory action. We can use them in the treatment of inflammation instead of NSAIDs and Steroids as we know that these two have greater side effects than the statin and the anti-oxidant (Abbas et al, 2014).
4. Another reason is that, we are observing the rat models using two types of data involving physiological and behavioural changes. So, we can observe which combination is best for both the physiological changes and behavioural changes among the four types of drug (Sali et al, 2012).

So, for the above mentions reasons, this study was performed. If this new treatment regimen can be established, it will be a great initiative in the treatment of inflammation.

# Chapter Six: Methodology and Materials

## 6.1 Animal

To perform the experiment, Wistar Rat was selected as animal model. The scientific name of the rat was Rattus norvegicus (Wistar Rat). The average age of the rat was 3 months (90 days), which is considered to be matured for the experiment purpose. The average weight of the rat was between 230gm - 250gm per rate. They were collected from the Jahangirnagar University (Pharmacology Laboratory) situated in Savar, Dhaka, Bangladesh. They were kept in a suitable room temperature ranging between $22^0$C- $25^0$C and also humidity was maintained between 60 ± 10%. The rat was kept in the dark and the light conditions in the 12Hour/Per Day cycle. So, there was no hindrance in their normal life style. Standard pellet chows were provided as their food and they were collected from Animal Resources Facility of ICDDR, BD (International Centre for Diarrhoeal Disease Research, Bangladesh) (Abbas et al, 2014).

**Figure 6.1: Rattus norvegicus (Wistar Rat)** (Nimse & Pal, 2015)

The food contained enough nutrients for the animals, so that they can stay in normal health condition. The water which was supplied for the rat definitely pure and served in the plastic container for drinking purpose (Filtered water). Total 120 rats were taken for the experiment purpose. Each group consisted of six rats and they were kept in separate cages. They were marked by using black marker pen. And identifying by numbering for an example, for number 1 rat, one single line was drawn in the tail of the rat and for

number 2 rat, double lines were drawn and so on. Before starting the experiment all rats were weighed by weighing machine individually. All trials were directed as per the Ethics Committee of the Department of Pharmacy, Jahangirnagar University. After the completion of the experiment, the rats were sacrificed with proper procedure.

### 6.2 Materials and reagents

#### 6.2.1 Distilled water

Distilled water was used for the rinsing purpose of the equipment. All equipment was rinsed with distilled water to avoid any kind of contamination.

#### 6.2.2 Normal Saline

0.9% Sodium Chloride Solution (Normal Saline) was used to dissolve the drug substances. The saline was packed in plastic bag. It was brought from Right Pharma Drug Shop, Mohakhali, Dhaka-1212, Bangladesh.

#### 6.2.3 Syringe

Insulin Syringe was used to administered drugs or other chemical substances in rats. The capacity of the syringes was 100 IU and total volume was 1ml. The syringe had 8mm needle, which was of very fine size and suitable for drug administration in rat model. Another syringe was used to collect the saline from the plastic bag with the volume of 50ml. All the syringes were bought from Right Pharma Drug Shop, Mohakhali, Dhaka-1212, Bangladesh.

#### 6.2.4 Cotton and Antiseptic agent

The cotton was used for rinsing the needle with antiseptic. Hexisol 50ml was used as an antiseptic agent that contains Chlorhexidine gluconate 0.5% w/v in Isopropyl alcohol BP 70%. It was used to rinse the syringe needle to avoid infection by microorganisms. Both the cotton and the Hexisol were bought from Right Pharma Drug Shop, Mohakhali, Dhaka-1212, Bangladesh and the manufacturer of Hexisol was ACI Ltd.

### 6.3 Inflammation inducing agents

There were two types of inflammation inducing agents used in the experiment-carrageenan and formalin.

### 6.3.1 Carrageenan

**Figure 6.2: Lambda Carrageenan Powder** (Sigma-Aldrich, 2017)

The alga *Chondrus crispus* is the principle constituent of carrageenan. 0.1ml freshly prepared 1% suspension of Carrageenan was used to induce local inflammation in the rat paw. The Lambda Carrageenan was used in the experiment as it has highest potency to induce inflammation rather than Kappa and Iota Carrageenan. 0.9% Sodium Chloride solution (Normal saline) was added to adjust the volume. It was prepared in a 100ml beaker. The Carrageenan was purchased from Sigma-Aldrich.

### 6.3.2 Formalin:

Formalin was also used to induce inflammation in rat which produces neurological effect in rat. 20µl of 5% formalin solution was injected beneath the footpad of the rats. At the beginning the rats elevated the injected paw and did not place it on the floor of the cage. The formalin produced its effects on rat in two phases. At the first phase, there was shaking and licking in the paw of the rats which lasts for 5 minutes. At the second phase, there was increased excitement in dorsal horn of neuron which lasts for 30 minutes. Activities like flinching, shaking, jerking and other abnormal activities were observed in the rats after formalin injection. 0.9% Sodium Chloride solution (Normal Saline) was added to adjust the volume of prepared formalin. The formalin (Formaldehyde) was purchased from the Viola Vitalis, House- #136/B, New DOHS, Mohakhali, Dhaka-1206, Bangladesh.

### 6.4 Drugs and Doses

There are four types drugs used for this study. They are Vitamin C, Atorvastatin, Diclofenac and Prednisolone. They all were administered in a definite dosage. All drugs were in pure form as active pharmaceutical ingredient. They were collected from the Incepta Pharmaceuticals Ltd, BD. There is a brief description about the dose of drugs used.

#### 6.4.1 Anti-Oxidant

Vitamin C (Ascorbic acid or L-ascorbic acid) was used as anti-oxidant. It was given in a concentration of about 200mg/kg and 100mg/kg body weight (Paiva et al, 2013).

#### 6.4.2 NSAID

Diclofenac was used as NSAIDs. It was given in a concentration about 5mg/kg body weight individually and with the combination of 100/mg/kg and 200mg/kg body weight vitamin c (ascorbic acid). It was dissolved in 0.9% Sodium chloride solution which is also known as normal saline (Silverstein et al, 2000).

#### 6.4.3 Steroid

The Prednisolone was used as steroid drug. It was given in a concentration about 5mg/kg body weight individually and also combined with the 100mg/kg and 200mg/kg body weight of Ascorbic acid. The prednisolone was dissolved in 0.9% normal saline in order to prepare for the administration (Kruif et al, 2007).

#### 6.4.4 Statin

Atorvastatin was used as the statin drug. It was given in a concentration about 8mg/kg body weight individually and with the combination of anti-oxidant (Vitamin C) in the concentration of 100mg/kg and 200mg/kg body weight. It was dissolved in the normal saline (0.9% NaCl solution). After that it was prepared for the intense purpose (Sparrow et al, 2000).

### 6.5 Group of Rats

The rats were divided into two groups- carrageenan and formalin group and the control group. The dose of drug for each group was same.

The following are the study groups of rats.

a) Vitamin C 100mg/kg body weight
b) Vitamin C 200mg/kg body weight
c) Diclofenac 5mg/kg body weight
d) Diclofenac 5mg/kg body weight with Vitamin C 100mg/kg body weight
e) Diclofenac 5mg/kg body weight with Vitamin C 200mg/kg body weight
f) Prednisolone 5mg/kg body weight
g) Prednisolone 5mg/kg body weight with Vitamin C 100mg/kg body weight
h) Prednisolone 5mg/kg body weight with Vitamin C 200mg/kg body weight
i) Atorvastatin 8mg/kg body weight
j) Atorvastatin 8mg/kg body weight with Vitamin C 100mg/kg body weight
k) Atorvastatin 8mg/kg body weight with Vitamin C 200mg/kg body weight

These were separate groups only for carrageenan, formalin and distilled water to observe their effects.

**6.6 Methodology**

1. All the rats were divided in several groups. Each group consisted of six rats (n=6) (Abbas et al, 2014).

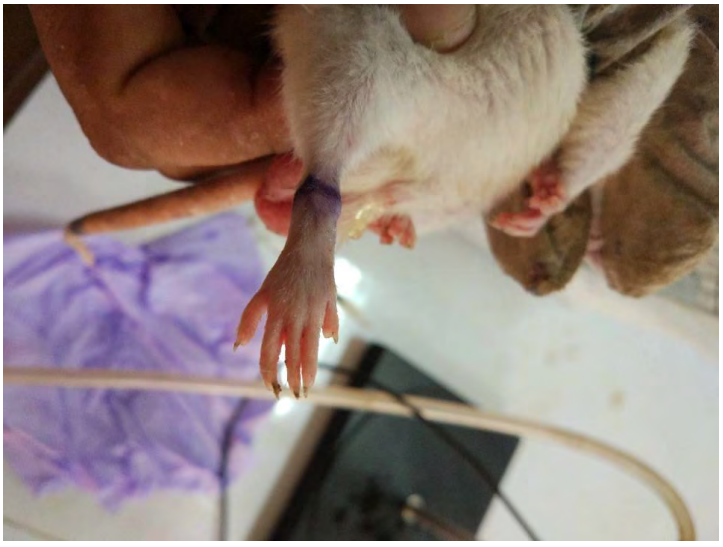

**Figure 6.3: Normal paw of Rat**

2. They were marked by black marker in their tail. For an example, the first rat in the group was marked by one single line in the rat and so on (Abbas et al, 2014).

3. At the beginning of the experiment, one group of rat was selected to induce inflammation by carrageenan and another group of rat was used to induce inflammation by formalin. No drugs administered (Abbas et al, 2014).

**Figure 6.4: Carrageenan induced paw edema in Rat**

4. First, drugs were administered 1 hour before carrageenan and formalin injection.

5. In case of carrageenan mediated inflammation group, the drugs were administered intaperitoneally.

6. In In case of formalin mediated inflammation group, the drugs were administered orally (Abbas et al, 2014).

**Figure 6.5: Licking activity of Rat**

7. The paw volume of the rat was measured by plethysmometer (a device to measure inflammation). After carrageenan injection, the paw volume of the rats was measured at 1st hour, 2nd hour, 3rd hour and 4th hour using the plethysmometer.

8. After the formalin injection, the number of licking by an individual rat was measured at 10 mins, 20 mins and 30 mins (Tabata et al, 2014).

### 6.7 Measurement of Inflammation using Plethysmometer

The Digital Water Plethysmometer is a profoundly valuable device for the estimation of little volume changes resulting inflammation. This test is regularly used to measure the advancement of the inflammatory reaction and to screen potential prohibition to oedema properties of pharmacological substances. Fundamentally, the volume transducer is framed by two Perspex tubes interconnected and loaded with a conductive arrangement and a platinum terminal for each chamber. The entire framework is upheld by a stand (incorporated) that can be put over the control unit (Sala et al, 2003).

**Figure 6.6: Plethysmometer**

The water dislodging delivered by the submersion of the rat paw in the measuring tube is reflected into the second tube, instigating an adjustment in the conductance between the two platinum cathodes. The Plethysmometer Control Unit recognizes the conductance

changes and creates a yield flag to the computerized show demonstrating the volume dislodging measured (0.01 ml determination). The present esteem stays in the advanced show until the point that another trial begins. The Control Unit is consequently focused between progressive readings, along these lines making halfway modifications pointless. A remote foot-switch permitting fast without hands analyses can be utilized to set control the end purpose of the estimation. (Sharma et al, 2004).

# Chapter Seven: Result and Observation

The experiment was done by using carrageenan and formalin to induce inflammation. So, there are two separate groups in the result. The first group involves carrageenan induced paw edema and the second group involves formalin induced inflammation.

## 7.1 Carrageenan mediated inflammation group

At first inflammation was induced by injecting carrageenan in the right hind paw of a rat, which produce paw edema. This value was considered as control value of paw edema. The paw edema volume was observed at 1st to 4th hour after injecting Carrageenan. Then, this is compared with the carrageenan mediated paw edema in the groups treated with drugs. The drugs were administered one hour before the carrageenan injection. After that, the percentage of inhibition was measured (Abbas et al, 2014). There were six rats per group. The formula for calculating the percentage of inhibition is

$$\text{PI} = \frac{Paw\ Volume\ of\ Control\ (Vt-V0) - Paw\ volume\ of\ Treated\ (Vt-V0)}{Paw\ Volume\ of\ Control\ (Vt-V0)} * 100$$

Where, $V_t$ = Paw volume at t time, $V_o$ = Paw volume at 0 time.

The following table represents our observation after the injection of carrageenan.

**Table 7.1: Standard Value of Paw edema of Rat after administration of Carrageenan**

| No. of Rat | $t_0$ Hour (ml) | 1 Hour (ml) | 2 Hour (ml) | 3 Hour (ml) | 4 Hour (ml) | Total paw edema |
|---|---|---|---|---|---|---|
| **1** | 0.76 | 1.02 | 1.44 | 1.56 | 1.58 | 2.56 |
| **2** | 0.69 | 1.25 | 1.78 | 2 | 2.35 | 4.62 |
| **3** | 0.72 | 1.34 | 1.89 | 2.2 | 2.55 | 5.1 |
| **4** | 0.72 | 1.12 | 1.77 | 2.25 | 2.45 | 4.71 |
| **5** | 0.77 | 1.35 | 1.82 | 1.95 | 2.69 | 4.73 |
| **6** | 0.75 | 1.31 | 1.79 | 2.13 | 1.98 | 4.21 |
| Average | | | | | | **4.32** |
| Standard Deviation | | | | | | **0.77** |

### 7.1.1 Anti-oxidant (100mg/kg) group

In this group, 100 mg/kg Vitamin C was administered intaperitoneally 1 hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.2: Percentage inhibition after IP administration of 100mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.66 | 38.00 | 45.54 | 14.06 | 1.31 | 19.68 |
| 2 | 0.78 | 38.00 | 43.56 | 12.50 | 0.65 | 18.52 |
| 3 | 0.92 | 38.00 | 43.56 | 11.72 | 0.65 | 18.29 |
| 4 | 0.65 | 36.00 | 43.56 | 13.28 | 1.96 | 18.98 |
| 5 | 0.84 | 38.00 | 42.57 | 13.28 | 1.31 | 18.75 |
| 6 | 0.99 | 38.00 | 44.55 | 14.06 | 1.96 | 19.68 |
| Average | | 37.67 | 43.89 | 13.15 | 1.31 | 18.98 |
| Standard Deviation | | 0.75 | 0.93 | 0.83 | 0.53 | 0.53 |

### 7.1.2 Anti-oxidant (200mg/kg) group

In this group, 200 mg/kg Vitamin C was administered by intaperitoneally 1 hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.3: Percentage inhibition after IP administration of 200mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.54 | 20.00 | 39.60 | 13.28 | 16.34 | 21.30 |
| 2 | 0.67 | 20.00 | 38.61 | 13.28 | 15.03 | 20.60 |
| 3 | 0.62 | 20.00 | 39.60 | 14.06 | 16.34 | 21.53 |
| 4 | 0.71 | 18.00 | 37.62 | 12.50 | 15.69 | 20.14 |
| 5 | 0.65 | 20.00 | 39.60 | 13.28 | 16.99 | 21.53 |
| 6 | 0.59 | 18.00 | 37.62 | 14.06 | 15.69 | 20.60 |
| Average | | 19.33 | 38.78 | 13.41 | 16.01 | 20.95 |
| Standard Deviation | | 0.94 | 0.89 | 0.54 | 0.63 | 0.53 |

### 7.1.3 NSAID (5mg/kg) group

In this group, the 5mg/kg Diclofenac was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.4: Percentage of inhibition after IP administration of 5mg/kg Diclofenac**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.91 | 64.00 | 49.50 | 39.06 | 28.10 | 40.51 |
| 2 | 0.82 | 64.00 | 50.50 | 39.84 | 27.45 | 40.74 |
| 3 | 0.75 | 66.00 | 50.50 | 39.06 | 26.80 | 40.51 |
| 4 | 0.95 | 66.00 | 48.51 | 39.06 | 26.14 | 39.81 |
| 5 | 0.88 | 66.00 | 50.50 | 40.63 | 27.45 | 41.20 |
| 6 | 0.65 | 66.00 | 49.50 | 39.84 | 26.14 | 40.28 |
| Average | | 65.33 | 49.83 | 39.58 | 27.02 | 40.51 |
| Standard Deviation | | 0.94 | 0.74 | 0.58 | 0.72 | 0.42 |

### 7.1.4 NSAID (5mg/kg) and Anti-oxidant (100mg/kg) group

In this group, a combination of 5mg/kg Diclofenac and 100mg/kg Vitamin C was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.5: Percentage of inhibition after IP administration of 5mg/kg Diclofenac and 100mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.75 | 72.92 | 62.24 | 62.40 | 63.81 | 45.77 |
| 2 | 0.71 | 70.83 | 62.24 | 63.23 | 63.81 | 46.74 |
| 3 | 0.73 | 72.92 | 63.27 | 63.23 | 63.81 | 46.74 |
| 4 | 0.84 | 70.83 | 61.22 | 64.07 | 64.50 | 43.81 |
| 5 | 0.76 | 72.92 | 61.22 | 63.23 | 63.81 | 45.52 |
| 6 | 0.79 | 72.92 | 62.24 | 63.23 | 64.50 | 45.28 |
| Average | | 72.22 | 62.07 | 63.23 | 64.04 | 45.64 |
| Standard Deviation | | 0.98 | 0.70 | 0.48 | 0.33 | 0.99 |

### 7.1.5 NSAID (5mg/kg) and Anti-oxidant (200mg/kg) group

In this group, a combination of 5mg/kg Diclofenac and 200mg/kg Vitamin C was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.6: Percentage of inhibition after IP administration of 5mg/kg Diclofenac and 200mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.76 | 62.50 | 75.51 | 71.59 | 79.12 | 74.10 |
| 2 | 0.98 | 62.50 | 74.49 | 71.59 | 78.42 | 73.62 |
| 3 | 1.03 | 64.58 | 75.51 | 72.42 | 79.81 | 74.84 |
| 4 | 0.78 | 64.58 | 76.53 | 70.75 | 79.12 | 74.35 |
| 5 | 0.71 | 64.58 | 74.49 | 71.59 | 78.42 | 73.86 |
| 6 | 0.84 | 64.58 | 75.51 | 71.59 | 79.12 | 74.35 |
| Average | | 63.89 | 75.34 | 71.59 | 79.00 | 74.19 |
| Standard Deviation | | 0.98 | 0.70 | 0.48 | 0.48 | 0.39 |

### 7.1.6 Steroid (5mg/kg) group

In this group, the 5mg/kg Prednisolone was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.7: Percentage of inhibition after IP administration of 5mg/kg Prednisolone**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.71 | 16.00 | 36.63 | 36.72 | 32.03 | 32.64 |
| 2 | 0.65 | 18.00 | 35.64 | 37.50 | 31.37 | 32.64 |
| 3 | 0.69 | 16.00 | 36.63 | 36.72 | 31.37 | 32.41 |
| 4 | 0.72 | 16.00 | 36.63 | 36.72 | 31.37 | 32.41 |
| 5 | 0.81 | 16.00 | 35.64 | 37.50 | 32.03 | 32.64 |
| 6 | 0.75 | 18.00 | 36.63 | 35.16 | 31.37 | 32.18 |
| Average | | 16.67 | 36.30 | 36.72 | 31.59 | 32.48 |
| Standard Deviation | | 0.94 | 0.47 | 0.78 | 0.31 | 0.17 |

### 7.1.7 Steroid (5mg/kg) and Anti-oxidant (100mg/kg) group

In this group, a combination of 5mg/kg Prednisolone and 100mg/kg Vitamin C was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.8: Percentage of inhibition after IP administration of 5mg/kg Prednisolone and 100mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 1.01 | 72.92 | 64.29 | 28.97 | 1.16 | 32.82 |
| 2 | 1.02 | 70.83 | 64.29 | 28.13 | 1.16 | 32.33 |
| 3 | 0.94 | 72.92 | 62.24 | 28.13 | 0.46 | 31.84 |
| 4 | 1.1 | 72.92 | 63.27 | 29.81 | 1.86 | 33.06 |
| 5 | 0.99 | 70.83 | 62.24 | 29.81 | 1.16 | 32.33 |
| 6 | 0.95 | 72.92 | 64.29 | 28.97 | 1.86 | 33.06 |
| Average | | 72.22 | 63.44 | 28.97 | 1.28 | 32.57 |
| Standard Deviation | | 0.98 | 0.92 | 0.68 | 0.48 | 0.45 |

### 7.1.8 Steroid (5mg/kg) and Anti-oxidant (200mg/kg) group

In this group, a combination of 5mg/kg Prednisolone and 200mg/kg Vitamin C was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.9: Percentage of inhibition after IP administration of 5mg/kg Prednisolone and 200mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.87 | 62.50 | 76.53 | 71.59 | 65.20 | 69.46 |
| 2 | 0.68 | 62.50 | 76.53 | 70.75 | 66.59 | 69.71 |
| 3 | 0.97 | 64.58 | 75.51 | 70.75 | 67.29 | 69.95 |
| 4 | 0.65 | 62.50 | 76.53 | 69.92 | 65.89 | 69.22 |
| 5 | 0.91 | 62.50 | 77.55 | 70.75 | 67.29 | 70.20 |
| 6 | 0.95 | 62.50 | 76.53 | 70.75 | 66.59 | 69.71 |
| Average | | 62.85 | 76.53 | 70.75 | 66.47 | 69.71 |
| Standard Deviation | | 0.78 | 0.59 | 0.48 | 0.74 | 0.32 |

### 7.1.9 Statin (8mg/kg) group

In this group, the (8mg/kg) Atorvastatin was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.10: Percentage of inhibition after IP administration of 8mg/kg Atorvastatin**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.6 | 38.00 | 22.77 | 22.66 | 18.95 | 23.15 |
| 2 | 0.89 | 36.00 | 23.76 | 23.44 | 19.61 | 23.61 |
| 3 | 0.75 | 38.00 | 23.76 | 23.44 | 19.61 | 23.84 |
| 4 | 0.68 | 36.00 | 21.78 | 21.88 | 20.26 | 22.92 |
| 5 | 0.81 | 38.00 | 22.77 | 22.66 | 19.61 | 23.38 |
| 6 | 0.83 | 38.00 | 22.77 | 23.44 | 19.61 | 23.61 |
| Average | | 37.33 | 22.94 | 22.92 | 19.61 | 23.42 |
| Standard Deviation | | 0.94 | 0.68 | 0.58 | 0.38 | 0.31 |

### 7.1.10 Statin (8mg/kg) and Anti-oxidant (100mg/kg) group

In this group, a combination of 8mg/kg Atorvastatin and 100mg/kg Vitamin C was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.11: Percentage of inhibition after IP administration of 8mg/kg Atorvastatin and 100mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.78 | 64.00 | 52.48 | 32.81 | 45.75 | 45.60 |
| 2 | 0.6 | 64.00 | 51.49 | 32.03 | 45.10 | 44.91 |
| 3 | 0.87 | 64.00 | 52.48 | 32.03 | 46.41 | 45.60 |
| 4 | 0.71 | 66.00 | 51.49 | 32.81 | 45.75 | 45.60 |
| 5 | 0.74 | 64.00 | 50.50 | 33.59 | 45.75 | 45.37 |
| 6 | 0.63 | 66.00 | 52.48 | 32.81 | 43.79 | 45.14 |
| Average | | 64.67 | 51.82 | 32.68 | 45.42 | 45.37 |
| Standard Deviation | | 0.94 | 0.74 | 0.54 | 0.82 | 0.27 |

### 7.1.11 Statin (8mg/kg) and Anti-oxidant (200mg/kg) group

In this group, a combination of 8mg/kg Atorvastatin and 200mg/kg Vitamin C was administered intaperitoneally one hour before the carrageenan injection. The observation is stated in the following table:

**Table 7.12: Percentage of inhibition after IP administration of 8mg/kg Atorvastatin and 200mg/kg Vitamin C**

| Rat No. | Initial Vol. (ml) | % of Inhibition at 1 hour | % of Inhibition at 2 hour | % of Inhibition at 3 hour | % of Inhibition at 4 hour | Total % of Inhibition |
|---|---|---|---|---|---|---|
| 1 | 0.66 | 54.17 | 63.27 | 51.53 | 37.36 | 49.67 |
| 2 | 0.71 | 56.25 | 64.29 | 50.70 | 37.36 | 49.92 |
| 3 | 0.76 | 56.25 | 64.29 | 51.53 | 38.05 | 50.41 |
| 4 | 0.99 | 56.25 | 63.27 | 51.53 | 38.05 | 50.16 |
| 5 | 0.86 | 56.25 | 64.29 | 52.37 | 38.75 | 50.90 |
| 6 | 0.94 | 54.17 | 64.29 | 51.53 | 38.05 | 50.16 |
| Average | | 55.56 | 63.95 | 51.53 | 37.94 | 50.20 |
| Standard Deviation | | 0.98 | 0.48 | 0.48 | 0.48 | 0.38 |

### 7.1.12 Overview of percentage of inhibition by the group of drugs in case of carrageenan mediated inflammation

Here, there is an overview about the anti-inflammatory actions as given by the drugs against inflammation mediated by carrageenan.

**Table 7.13: The anti-inflammatory actions observed against carrageenan induced rat paw inflammation**

| SL No. | Group Name | Percentage of Inhibition (%) | Standard Deviation |
|---|---|---|---|
| **1** | Anti-oxidant (100mg/kg) | 18.98 | ± 0.53 |
| **2** | Anti-oxidant (200mg/kg) | 20.95 | ± 0.53 |
| **3** | NSAID (5mg/kg) | 40.51 | ± 0.42 |
| **4** | NSAID (5mg/kg) + Anti-oxidant (100mg/kg) | 45.64 | ± 0.99 |
| **5** | NSAID (5mg/kg) + Anti-oxidant (200mg/kg) | 74.19 | ± 0.39 |
| **6** | Steroid (5mg/kg) | 32.48 | ± 0.17 |
| **7** | Steroid (5mg/kg) + Anti-oxidant (100mg/kg) | 32.57 | ± 0.45 |
| **8** | Steroid (5mg/kg) + Anti-oxidant (200mg/kg) | 69.71 | ± 0.32 |
| **9** | Statin (8mg/kg) | 23.42 | ± 0.31 |
| **10** | Statin (8mg/kg) + Anti-oxidant (100mg/kg) | 45.37 | ± 0.27 |
| **11** | Statin (8mg/kg) + Anti-oxidant (200mg/kg) | 50.2 | ± 0.38 |

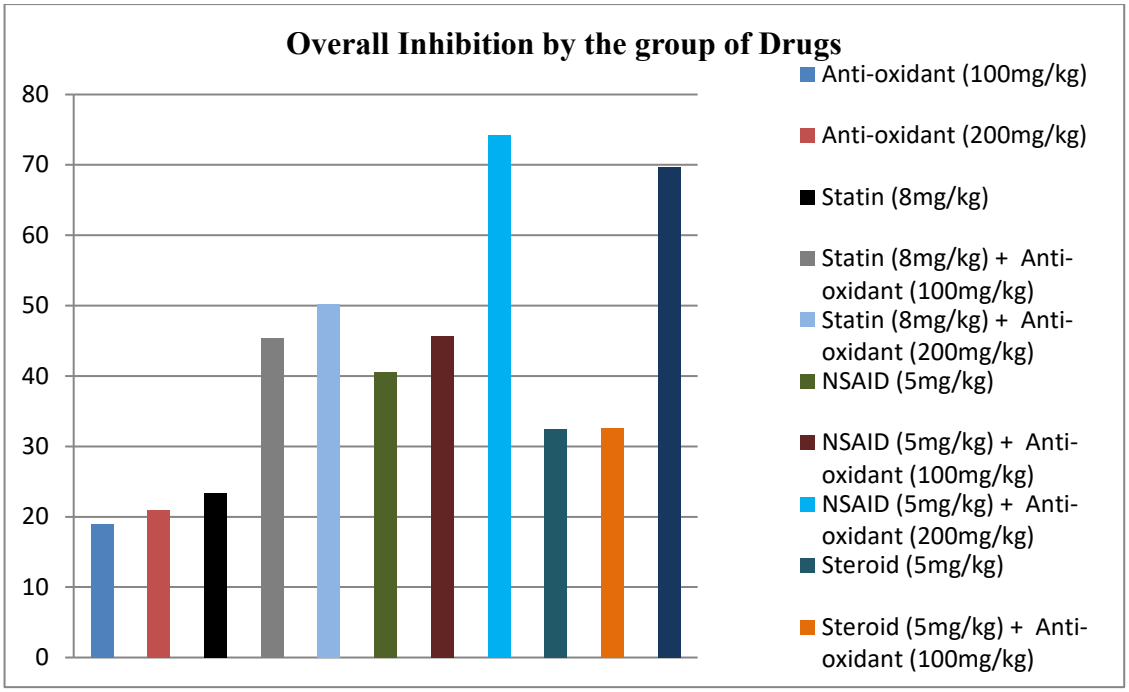


**Figure 7.1: The graphical presentation of percentage of inhibition by all groups of drug**

### 7.2 Formalin Mediated Inflammation Group

The inflammation was produced by formalin injection in the right hind paw of the rats. The formalin generally produces neurological effect like licking, itching, flinching, shaking and jerking etc. like activity. In this experiment, the abnormal reactions of the rats were observed. The value of the formalin mediated licking without drug is considered as control group. The number of licking activity was observed for 10 minutes, then for next 10 min and finally last 10 min for the total period of 30 min. Then, the number of licking by the rats by formalin injection is compared with the number of licking of the rats in the groups which were treated with drug one hour before formalin injection. After that, the percentage of inhibition was measured by compared with the standard group (formalin mediated licking).

#### 7.2.1 Formalin group

In this group, 20 micro-litre 5% formalin solutions were injected in the right hind paw of the rats. After that, the rats were licking their paw. The number of licking by the rats was observed at 0 minutes, 10 minutes, 20 minutes and 30 minutes consecutively. This group is considered as control group (Mecarson, 1999).

The formula of the percentage of inhibition is given as follow:

$$\text{PI} = \frac{Number\ of\ paw\ licking\ in\ Control - Number\ of\ paw\ licking\ in\ treated}{Number\ of\ paw\ licking\ in\ control} * 100$$

Now, the observations and results are given below.

Let's see the table given below:

**Table 7.14: Number of Licking by rats after administration of formalin**

| No. of Rat | 10 min | 20 min | 30 min | Total Licking |
|---|---|---|---|---|
| 1 | 58 | 3 | 0 | 61 |
| 2 | 55 | 4 | 2 | 61 |
| 3 | 56 | 3 | 2 | 61 |
| 4 | 53 | 5 | 1 | 59 |
| 5 | 60 | 1 | 0 | 61 |
| 6 | 49 | 8 | 4 | 61 |
| Average | 55.17 | 4 | 1.5 | 60.67 |
| Standard Deviation | | | | 0.75 |

### 7.2.2 Anti-oxidant (100mg/kg) group

In this group, 100mg/kg Vitamin C was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.15: Percentage of inhibition after oral administration of 100mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 3 | 0 | 0 | 3 | 60.67 | 95.06 |
| 2 | 4 | 0 | 0 | 4 | 60.67 | 93.41 |
| 3 | 3 | 0 | 0 | 3 | 60.67 | 95.06 |
| 4 | 4 | 0 | | 4 | 60.67 | 93.41 |
| 5 | 3 | 0 | 0 | 3 | 60.67 | 95.06 |
| 6 | 3 | 0 | 0 | 3 | 60.67 | 95.06 |
| Average | | | | | | 94.51 |
| Standard Deviation | | | | | | 0.78 |

### 7.2.3 Anti-oxidant (200mg/kg) group

In this group, 200mg/kg Vitamin C was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.16: Percentage of inhibition after oral administration of 200mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 2 | 0 | 0 | 2 | 60.67 | 96.70348 |
| 2 | 2 | 0 | 0 | 2 | 60.67 | 96.70348 |
| 3 | 1 | 0 | 0 | 1 | 60.67 | 98.35174 |
| 4 | 2 | 0 | 0 | 2 | 60.67 | 96.70348 |
| 5 | 1 | 0 | 0 | 1 | 60.67 | 98.35174 |
| 6 | 1 | 0 | 0 | 1 | 60.67 | 98.35174 |
| Average | | | | | | 97.53 |
| Standard Deviation | | | | | | 0.824131 |

### 7.2.4 NSAID (5mg/kg) group

In this group, 5mg/kg Diclofenac was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.17: Percentage of inhibition after oral administration of 5mg/kg Diclofenac**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 33 | 0 | 5 | 38 | 60.67 | 37.37 |
| 2 | 39 | 0 | 0 | 39 | 60.67 | 35.72 |
| 3 | 38 | 0 | 2 | 40 | 60.67 | 34.07 |
| 4 | 37 | 0 | 0 | 37 | 60.67 | 39.01 |
| 5 | 40 | 0 | 0 | 40 | 60.67 | 34.07 |
| 6 | 35 | 0 | 4 | 39 | 60.67 | 35.72 |
| Average | | | | | | 35.99 |
| Standard Deviation | | | | | | 1.76 |

### 7.2.5 NSAID (5mg/kg) and Anti-oxidant (100mg/kg) group

In this group, 5mg/kg Diclofenac and 100mg/kg Vitamin C body was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.18: Percentage of inhibition after oral administration of 5mg/kg Diclofenac and 100mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 0 | 3 | 0 | 3 | 60.67 | 95.05 |
| 2 | 0 | 4 | 0 | 4 | 60.67 | 93.40 |
| 3 | 0 | 3 | 1 | 4 | 60.67 | 93.40 |
| 4 | 3 | 0 | 1 | 4 | 60.67 | 93.40 |
| 5 | 0 | 5 | 0 | 5 | 60.67 | 91.75 |
| 6 | 0 | 4 | 0 | 4 | 60.67 | 93.40 |
| Average | | | | | | 93.41 |
| Standard Deviation | | | | | | 0.95 |

### 7.2.6 NSAID (5mg/kg) and Anti-oxidant (200mg/kg) group

In this group, 5mg/kg Diclofenac and 200mg/kg Vitamin C body was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.19: Percentage of inhibition after oral administration of 5mg/kg Diclofenac and 200mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 0 | 2 | 0 | 2 | 60.67 | 96.70 |
| 2 | 0 | 0 | 3 | 3 | 60.67 | 95.05522 |
| 3 | 0 | 0 | 0 | 0 | 60.67 | 100 |
| 4 | 0 | 0 | 2 | 2 | 60.67 | 96.70 |
| 5 | 0 | 0 | 1 | 1 | 60.67 | 98.35 |
| 6 | 0 | 0 | 2 | 2 | 60.67 | 96.70348 |
| Average | | | | | | 97.25 |
| Standard Deviation | | | | | | 1.55 |

### 7.2.7 Steroid (5mg/kg) group

In this group, 5mg/kg Prednisolone was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.20: Percentage of inhibition after oral administration of 5mg/kg Prednisolone**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 29 | 0 | 0 | 29 | 60.67 | 52.20 |
| 2 | 27 | 0 | 0 | 27 | 60.67 | 55.50 |
| 3 | 30 | | 0 | 30 | 60.67 | 50.55 |
| 4 | 28 | | 0 | 28 | 60.67 | 53.85 |
| 5 | 27 | | 0 | 27 | 60.67 | 55.50 |
| 6 | 28 | | 0 | 28 | 60.67 | 53.85 |
| Average | | | | | | 53.57 |
| Standard Deviation | | | | | | 1.76 |

### 7.2.8 Steroid (5mg/kg) and Anti-oxidant (100mg/kg)

In this group, 5mg/kg Prednisolone and 100mg/kg Vitamin C was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.21: Percentage of inhibition after oral administration of 5mg/kg Prednisolone and 100mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 23 | 0 | | 23 | 60.67 | 62.09 |
| 2 | 23 | 0 | 0 | 23 | 60.67 | 62.09 |
| 3 | 24 | 0 | 0 | 24 | 60.67 | 60.44 |
| 4 | 24 | 0 | 0 | 24 | 60.67 | 60.44 |
| 5 | 23 | 0 | 0 | 23 | 60.67 | 62.09 |
| 6 | 24 | 0 | 0 | 24 | 60.67 | 60.44 |
| Average | | | | | | 61.27 |
| Standard Deviation | | | | | | 0.82 |

### 7.2.9 Steroid (5mg/kg) and Anti-oxidant (200mg/kg)

In this group, 5mg/kg Prednisolone and 200mg/kg Vitamin C was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.22: Percentage of inhibition after oral administration of 5mg/kg Prednisolone and 200mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 7 | 9 | 0 | 16 | 60.67 | 73.63 |
| 2 | 16 | 0 | 0 | 16 | 60.67 | 73.63 |
| 3 | 15 | 0 | 0 | 15 | 60.67 | 75.28 |
| 4 | 9 | 0 | 7 | 16 | 60.67 | 73.63 |
| 5 | 0 | 0 | 15 | 15 | 60.67 | 75.28 |
| 6 | 8 | 0 | 8 | 16 | 60.67 | 73.63 |
| Average | | | | | | 74.18 |
| Standard Deviation | | | | | | 0.78 |

### 7.2.10 Statin (8mg/kg) group

In this group, 8mg/kg Atorvastatin body weight was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.23: Percentage of inhibition after oral administration of 8mg/kg Atorvastatin**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 32 | 0 | 0 | 32 | 60.67 | 47.26 |
| 2 | 29 | 0 | 0 | 29 | 60.67 | 52.20 |
| 3 | 29 | 1 | 1 | 31 | 60.67 | 48.90 |
| 4 | 29 | 0 | 2 | 31 | 60.67 | 48.90 |
| 5 | 31 | 0 | 0 | 31 | 60.67 | 48.90 |
| 6 | 25 | 0 | 7 | 32 | 60.67 | 47.26 |
| Average | | | | | | 48.90 |
| Standard Deviation | | | | | | 1.65 |

### 7.2.11 Statin (8mg/kg) and Anti-oxidant (100mg/kg) group

In this group, 8mg/kg Atorvastatin and 100mg/kg Vitamin C was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.24: Percentage of inhibition after oral administration of 8mg/kg Atorvastatin and 100mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 15 | 0 | 15 | 30 | 60.67 | 50.55 |
| 2 | 28 | | 3 | 31 | 60.67 | 48.90 |
| 3 | 20 | 0 | 9 | 29 | 60.67 | 52.20 |
| 4 | 17 | 0 | 12 | 29 | 60.67 | 52.20 |
| 5 | 25 | 0 | 5 | 30 | 60.67 | 50.55 |
| 6 | 18 | 0 | 13 | 31 | 60.67 | 48.90 |
| Average | | | | | | 50.55 |
| Standard Deviation | | | | | | 1.35 |

### 7.2.12 Statin (8mg/kg) and Anti-oxidant (200mg/kg) group

In this group, 8mg/kg Atorvastatin and 200mg/kg Vitamin C was administered orally one hour before the formalin injection. The observation is stated in the following table:

**Table 7.25: Percentage of inhibition after oral administration of 8mg/kg Atorvastatin and 200mg/kg Vitamin C**

| Rat No. | Number of Licking at 10 min | Number of Licking at 20 min | Number of Licking at 30 min | Total Number of Licking | Standar value | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 8 | 0 | 0 | 8 | 60.67 | 86.81 |
| 2 | 9 | 0 | 0 | 9 | 60.67 | 85.17 |
| 3 | 3 | 0 | 5 | 8 | 60.67 | 86.81 |
| 4 | 8 | 0 | 0 | 8 | 60.67 | 86.81 |
| 5 | 5 | 0 | 4 | 9 | 60.67 | 85.17 |
| 6 | 8 | 0 | 0 | 8 | 60.67 | 86.81 |
| Average | | | | | | 86.26 |
| Standard Deviation | | | | | | 0.78 |

### 7.2.13 Overview of the percentage of inhibition by the group of drugs in case of formalin mediated inflammation

Here, there is an overview about the anti-inflammatory actions are given by the drugs, where inflammation was mediated by formalin injection.

**Table 7.26: The anti-inflammatory actions observed against formalin induced neuropsychiatric effects on rats**

| SL No. | Group Name | Percentage of Inhibition (%) | Standard Deviation |
|---|---|---|---|
| **1** | Anti-oxidant (100mg/kg) | 94.51 | ± 0.78 |
| **2** | Anti-oxidant (200mg/kg) | 97.53 | ± 0.82 |
| **3** | NSAID (5mg/kg) | 35.99 | ± 1.76 |
| **4** | NSAID (5mg/kg) + Anti-oxidant (100mg/kg) | 93.41 | ± 0.95 |
| **5** | NSAID (5mg/kg) + Anti-oxidant (200mg/kg) | 97.25 | ± 1.55 |
| **6** | Steroid (5mg/kg) | 53.57 | ± 1.76 |
| **7** | Steroid (5mg/kg) + Anti-oxidant (100mg/kg) | 61.27 | ± 0.82 |
| **8** | Steroid (5mg/kg) + Anti-oxidant (200mg/kg) | 74.18 | ± 0.78 |
| **9** | Statin (8mg/kg) | 48.90 | ± 1.65 |
| **10** | Statin (8mg/kg) + Anti-oxidant (100mg/kg) | 50.55 | ± 1.35 |
| **11** | Statin (8mg/kg) + Anti-oxidant (200mg/kg) | 86.26 | ± 0.78 |

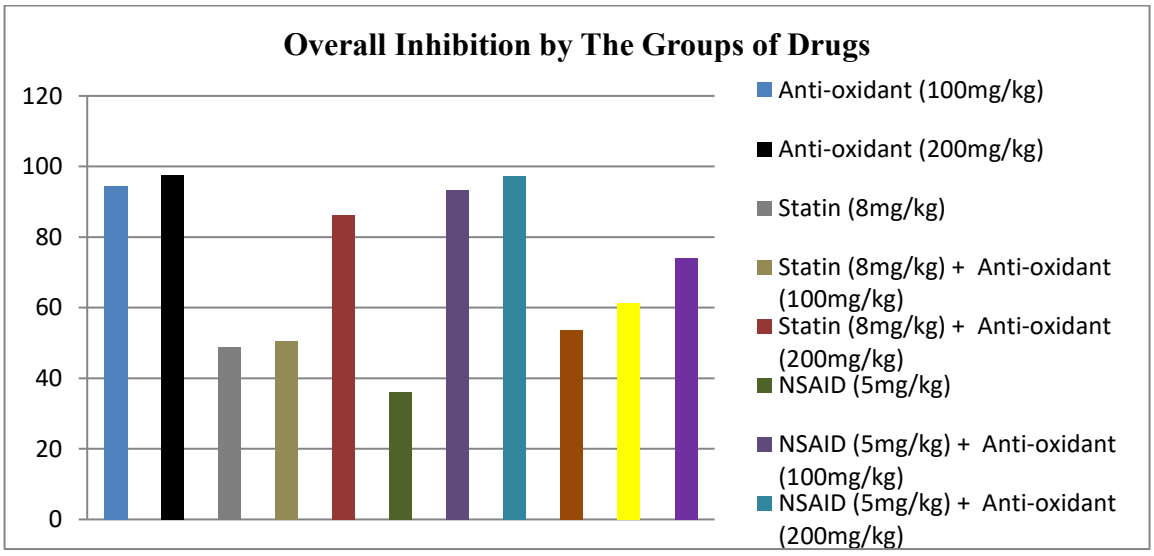


**Figure 7.2: The graphical presentation of percentage of inhibition by all groups of drug**

## Chapter Eight: Discussion

In acute inflammation, migration of granulocytes is greatly increased; mainly neutrophils followed by monocytes which later mature to phagocytic macrophages. Tissue mediators such as oxygen radicals, histamine, serotonin, kinins, eonosinophil chemotactic factor and components of compliment cause the inflammatory response. Metabolites arising from Arachidonic acid modulate other potential modulators in acute inflammatory response. They are termed eicosanoids and include prostaglandins, thromboxane and leukotrienes, as well as several hydroxyacids. Prostaglandins are one of the more potent mediators that cause increased blood flow, chemotaxis (chemical signals that summon white blood cells), and subsequent dysfunction of tissues and organs. They are the response of the immune system to injurious agents and prostaglandins will continue to add to the inflammatory process as long as the injurious agents persist (Cassell, 1998).

Prostaglandins are formed through metabolism of arachidonic acid by cyclooxygenase (COX) enzymes and other sequential synthases. The formation of prostaglandins depend on the activity of cyclooxygenases which exist as COX-1 and COX-2 isoforms. COX-1 promotes prostaglandin formation in most cells as homeostatic function and mainly COX-2 promotes prostaglandin formation in inflammation after adverse stimuli (Ricciotti & Fitzgerald, 2011).

Another contributor to inflammation is oxidative stress which is the imbalance of excessive reactive oxygen species (ROS) production and their neutralization by defense mechanism like the antioxidant system. ROS production is greatly increased during inflammation by phagocytic cells. Oxidative stress can induce inflammation by activating transcription factors which lead to deferential gene expressions. By activating transcription factor NF-κB hydrogen peroxide can cause inflammation. Oxidative stress also plays an important role in the activation of NOD-like receptor protein 3 (NLRP3) inflammasome, which is an oligomeric molecular complex that triggers innate immune defenses through the maturation of proinflammatory cytokines like IL-1β and IL-18. Moreover, oxidative stress can cause damage to all major cellular components such as DNA, proteins and lipids and may lead to cell death. Inflammation can also occur due to DNA base modification induced by ROS (Hussain et al, 2016).

The use of carrageenan to induce acute inflammation in rats is an established test to evaluate the effects of anti- inflammatory drugs and their anti-edematous effects.

Carrageenan stimulates the release of inflammatory mediators such as prostaglandins, leukotrienes, histamine, bradykinin, TNF-α, etc. The induced local inflammation is a biphasic process; first phase starting with histamine, serotonin, and kinins being released in the initial hour. Prostaglandins are released in the second phase in the later hours which are mainly responsible for the inflammation (Mcginnis & Madden, 2004).

Formalin is an irritant causing inflammation and pain behavior in rats. This is also a biphasic process initiating with licking and biting in the first 5-10 minutes and pain behavior of 30 minutes after a 5-15 minutes of normal behavior. The first phase response is assumed to be due to direct activation of nociceptive neurons by formalin and tissue injury causes the second phase inflammatory response. Bradykinins play a major role in formalin induced inflammation. Bradykinins increase production of ROS through activation of $B_2$-kinin receptor (Greene et al, 2000).

Antioxidants are oxidation inhibiting substances. They defend our body against oxidative stress. They neutralize excessive reactive species and retain the balance in between and also act as scavengers for free radicals. As mentioned above, inflammation can be caused by oxidative stress and antioxidants also play a contributing role in modulating inflammation by reducing reactive species and free radicals thus, controlling oxidative stress (Kass, 2001).

We have administered antioxidant Vitamin C as monotherapy in the rats to assess their effect in inhibiting inflammation induced by carrageenan and formalin. Two groups of rats were given doses of 100mg and 200mg Vitamin C for each of the inflammatory agents. For carrageenan, the group given 100 mg Vitamin C showed 37.67%, 43.89%, 13.15% and 1.31% of inhibition consecutively each hour up to 4 hours with standard deviations of 0.75, 0.93, 0.83 and 0.53 respectively. The average inhibition was 18.98% with 0.27 standard deviation. The results were 19.33%, 38.78%, 13.41% and 16.01% of inhibition after each hour consecutively up to 4 hours with standard deviations of 0.94, 0.89, 0.54 and 0.63 respectively for the other group given 200 mg of Vitamin C. The average inhibition was 20.95% with 0.53 standard deviation. The higher dose clearly shows slightly elevated inhibition. For formalin induced inflammation, the group given 100 mg of Vitamin C showed 94.51% of inhibition on average and 97.53% inhibition on average for the group given 200 mg of Vitamin C (Kass, 2001).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common choices of medication in dealing with acute inflammation and pain. NSAIDs elicit their anti-inflammatory action by inhibiting the cyclooxygenase (COX) and thus preventing prostaglandin synthesis and other eicosanoids. Inhibition of prostaglandin synthesis disrupts the inflammatory response and mitigates inflammation and pain (Osafo et al, 2017). We have administered 5 mg/kg of Diclofenac Sodium, a NSAID to the rat groups for both carrageenan and formalin induced inflammation as monotherapy and also in combination with antioxidant Vitamin C. The antioxidant was given in two separate doses of 100 mg and 200 mg. In case of carrageenan, the monotherapy of NSAID showed 65.33%, 49.83%, 39.58% and 27.02% of inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours. The average inhibition was 40.51%. However, in combination therapy with 100 mg antioxidant, the results were 72.22%, 62.07%, 63.23% and 64.04% of inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours and an average of 45.64% inhibition. For combination therapy with 200 mg of antioxidant, the results were 63.89%, 75.34%, 71.59% and 79% of inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours and an average of 74.19% of inhibition. In case of formalin, the monotherapy of NSAID showed an average of 35.99% inhibition. However, in combination therapy with 100 mg antioxidant the inhibition was 93.41 % and with 200 mg antioxidant the inhibition was 97.25%. Clearly, in both cases of carrageenan and formalin induced inflammation the combination therapy with antioxidant Vitamin C showed much better results for NSAID and the higher dose of antioxidant gave better results.

In addition to their lipid lowering effects, statins have also been found to demonstrate anti-inflammatory effects. They elicit their anti-inflammatory effects by reducing circulating C-reactive proteins (CRP) levels and pro-inflammatory cytokines. They also decrease vascular ROS production. Furthermore, statins also cause reduced macrophage expression of soluble intercellular adhesion molecule-1 and lipopolysaccharide-induced secretion of IL-6 and TNF-α by monocytes and macrophages (Antonopoulos et al, 2012). We have administered 8 mg/kg of Atorvastatin to the rat groups for both carrageenan and formalin induced inflammation in monotherapy as well as with two doses of antioxidant Vitamin C. For carrageenan, the monotherapy of Atorvastatin showed 37.33%, 22.94%, 22.92% and 19.61% of inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours and an average of 23.42% inhibition. However, when used in combination therapy with

100 mg antioxidant the results were 64.67%, 51.82%, 32.68% and 45.42% inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours and the average inhibition was 45.37%. For combination therapy of Atorvastatin with 200 mg of antioxidant the results were 55.56%, 63.95%, 51.53% and 37.94% after respectively 1 hour, 2 hours, 3 hours and 4 hours and an average of 50.20% inhibition. In case of formalin, the monotherapy of Atorvastatin showed an average of 48.90% inhibition. However, in combination therapy with 100 mg antioxidant the inhibition was 50.55 % and with 200 mg antioxidant the inhibition was 86.26%. The combination therapy showed better results than the monotherapy with the higher dose of antioxidant showing better results (Saleem & Basha, 2010).

Studies have established the anti-inflammatory effects of steroids. Steroids inhibit prostaglandin synthesis by inducing biosynthesis of phospholipase $A_2$ inhibitor (Flower & Blackwell, 1979). They decrease leukocyte migration to inflamed cells. Glucocorticoids repress transcription of many genes encoding pro-inflammatory cytokines and chemokines, cell adhesion molecules and key enzymes involved in the initiation and/or maintenance of the host inflammatory response (Coutinho & Chapman, 2011). We have administered 5 mg/kg of Prednisolone to the rat groups for both carrageenan and formalin induced inflammation in monotherapy as well as with antioxidant Vitamin C of 100 mg and 200 mg doses. For carrageenan, the monotherapy of Prednisolone showed 16.67%, 36.30%, 36.72% and 31.59% of inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours and an average of 32.48% inhibition. However, when used in combination therapy with 100 mg antioxidant the results were 72.22%, 63.44%, 28.97% and 1.28% inhibition after respectively 1 hour, 2 hours, 3 hours and 4 hours and the average inhibition was 32.57%. For combination therapy of Prednisolone with 200 mg of antioxidant the results were 62.85%, 76.53%, 70.75% and 66.47% after respectively 1 hour, 2 hours, 3 hours and 4 hours and an average of 69.71% inhibition. In case of formalin, the monotherapy of Prednisolone showed an average of 53.57% inhibition. However, in combination therapy with 100 mg antioxidant the inhibition was 61.27 % and with 200 mg antioxidant the inhibition was 74.18%. The combination therapy with 100 mg antioxidant showed slightly elevated inhibition than the monotherapy. However, the combination therapy with 200 mg antioxidant showed much better results.

**Table 8.1: Summary of anti-inflammatory effect of drug for both carrageenan and formalin induced inflammation**

| Therapy | Carrageenan | Formalin |
|---|---|---|
| **Antioxidant 100 mg** | 18.98% ±0.53 | 94.51%±0.78 |
| **Antioxidant 200 mg** | 20.95%±0.53 | 97.53%±0.824 |
| **NSAID** | 40.51%±0.42 | 35.99%±1.76 |
| **NSAID+ Antioxidant 100 mg** | 45.64%±0.99 | 93.41%±0.95 |
| **NSAID+ Antioxidant 200 mg** | 74.19%±0.39 | 97.25%±1.55 |
| **Steroid** | 32.48%±0.17 | 53.57%±1.76 |
| **Steroid+ Antioxidant 100 mg** | 32.57%±0.45 | 61.27%±0.82 |
| **Steroid+ Antioxidant 200 mg** | 69.71%±0.32 | 74.18%±0.78 |
| **Statin** | 23.42%±0.31 | 48.90%±1.65 |
| **Statin+ Antioxidant 100 mg** | 45.37%±0.27 | 50.55%±1.35 |
| **Statin+ Antioxidant 200 mg** | 50.20%±0.38 | 86.26%±0.78 |

From the table we can see that, in case of carrageenan induced inflammation, the best result was showed by the combination therapy of NSAID and 200 mg antioxidant with 74.19% inhibition. The combination therapy of Prednisolone and 200 mg antioxidant came in second with 69.71% inhibition. The better performance of NSAID combination therapy than Prednisolone combination therapy is due to the fact that NSAID's work on the molecular level by inhibiting COX and halting prostaglandin synthesis and in carrageenan induced inflammation, there are increased expression of COX 2 and PGE2 (Kass, 2001). Prostaglandins are produced in the injured cells and result in inflammatory response very quickly after the injurious stimuli and inhibiting the synthesis of prostaglandins give a quick onset of anti-inflammatory effect. However, steroids work on the genetic level by repressing transcription of several genes which take a longer time to take effect as it is a longer route of action. In both cases, addition of antioxidant potentiated their actions. The poorest results were obtained from antioxidant monotherapy as it works against only oxidative stress and cannot suppress other mediators of inflammation. Combination therapy of NSAID and 200 mg of antioxidant showed gradual increase in effectiveness and the best results was obtained in the fourth hour and combination therapy of NSAID and 100 mg antioxidant showed best effects in the first hour and then in the fourth hour. NSAID monotherapy gave peak performance in the first hour and then gradually declined. Effect of steroid monotherapy gradually

increased till the third hour and then declined. Steroid combined with 100 mg antioxidant gave peak performance in the first hour and then kept declining. However, in combination with 200 mg antioxidant steroid's effect was greatest in the second hour and then it kept declining. For statin monotherapy and combination therapy with antioxidant the best effects were in the first hour and then it gradually declined. Antioxidant monotherapy gave the best results in the first two hours and the peak performance in the second hour and then the efficacy declined. As for formalin induced inflammation, the monotherapy of antioxidants showed best results. The combination therapy of NSAID also showed excellent results however, the monotherapy of NSAID showed the poorest result. Atorvastatin and Prednisolone both showed moderate results in both cases of monotherapy and combination therapy however, the results were better in case of combination therapy. The exceeding performance of NSAID's is because of their inhibitory effect on the COX pathway as it is a major component of the inflammatory response. Between carrageenan and formalin induced inflammation, better inhibition was obtained in case of formalin. This is because carrageenan is a stronger inflammatory agent than formalin as there are several inflammatory mediators involved in carrageenan induced inflammation such as prostaglandins, serotonin, and histamine. However, in case of formalin the main contributors are bradykinin and reactive oxygen species and due to this reason the results were much better regarding formalin induced inflammation as there are less mediators to suppress. NSAID's were highly effective in both cases of carrageenan and formalin due to its effect on the COX pathway and it cannot be replaced neither by statins nor antioxidant monotherapy as their effects were not that prominent.

## Chapter Ten: Conclusion

To conclude, the study shows alternative drugs for the treatment of inflammatory diseases. At present inflammatory diseases have become a major issue (Tenner & Pisalyaput, 2011). The conventional anti-inflammatory agents have many side-effects and there is harmful effect on long term use of these types of drugs. So, to get rid of those problems establishing a new drug regimen has become necessary. In this study, antioxidants have been used to increase the therapeutic effects of conventional anti-inflammatory drugs like NSAID and steroid. Diclofenac was used as NSAID and Prednisolone was used as steroidal agent. Atorvastatin was also used in this study as it is a newly established anti-inflammatory drug. Inflammation was induced by using formalin and carrageenan injection. Carrageenan gave local inflammatory effects on rat paw and formalin gave neurological effects on rats. The percentage of inhibition of inflammation on rats was measured in order to observe the anti-inflammatory effects of drugs. It was found that, in case of carrageenan mediated inflammation, the combination of Diclofenac and higher dose of Vitamin C have shown the most percentage inhibition of inflammation. Similarly, in case of formalin mediated inflammation, the combination of Diclofenac and higher dose of Vitamin C have given the most inhibition and also higher dose of Vitamin C itself gave a greater inhibition of neuropsychiatric effect. This indicates that antioxidant have anti-inflammatory property in case of neurological diseases. Thus, antioxidants have actually potentiated the anti-inflammatory effects of the conventional anti-inflammatory drugs. There were some challenges to perform this study. We could not measure the level of inflammatory markers and the effect of drugs on them because of technical reasons. However, we have tried to make the best use of our available facilities. We also have plans to carry on this work for seeing the wound healing effect of these combinations. We hope that the result from our study, even though in small scale, will contribute in bringing changes to the management of anti-inflammatory disorders.

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