To investigate the Preventive and Therapeutic Role of Zingerone in Adjuvant-induced Arthritis in Experimental Rats

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(2017-V-354-M)

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Thesis

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Faculty of Veterinary Sciences
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Master of Veterinary Science
(Division of Veterinary Biochemistry)

2020

Dedicated

TO

MY beloved parents

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Veterinary Biochemistry
Shuhama Campus, Srinagar-190006

Certificate – I

This is to certify that the thesis entitled, "**To investigate the Preventive and Therapeutic Role of Zingerone in Adjuvant-induced Arthritis in Experimental Rats "** submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Sciences (Veterinary Biochemistry) to the Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Dr. Nazirah Bashir (Regd. No. 2017-V-354-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that information received during the course of investigation has dully been acknowledged.

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Certificate – III

This is to certify that the thesis entitled, **"To investigate the Preventive and Therapeutic Role of Zingerone in Adjuvant-induced Arthritis in Experimental Rats"** submitted by Dr. Nazirah Bashir (Regd. No. 2017-V-354-M), to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfillment of the requirements for the degree of **Master in Veterinary Sciences (Veterinary Biochemistry)** was examined and approved by the Advisory Committee and external examiner on_____

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that preferentially affects the synovial membranes of joints and eventually leads to bone and cartilage destruction. Identification of common dietary substances capable of affording protection or modulating the onset and severity of arthritis may have important health implications. Zingerone (ZGR), a phenolic alkanone isolated from ginger, has been reported to possess various pharmacological activities. Current study was designed to investigate the protective effect of zingerone in complete Freund's adjuvant (CFA) inoculated rats. Wistar albino rats (150–200g) of either sex were used for the experiment. Animals in group I served as a control group, group II as arthritic group, Group III as arthritic treated with zingerone for 21 days at the dose rate of 25 mg/kg b.wt., from the day of arthritis induction, Group IV as arthritic treated with zingerone for 21 days at the dose rate of 25 mg/kg b.wt., from the day of arthritis onset . Zingerone induced anti-arthritic activity was

carried out by using prophylactic model and therapeutic model. Arthritis was induced by single intra-dermal injection of 0.1ml of Complete Freund's Adjuvant at the base of tail**.** The protective effects of zingerone on CFA -induced oxidative stress and inflammation were investigated by assaying oxidative stress biomarkers, lipid peroxidation, serum inflammatory marker(CRP), levels of nuclear factor kappa B (NFκB), tumor necrosis factor alpha (TNF-α), transforming growth factor (TGF-β), interleukin-1β ,interleukin-6 and interleukin-10. CFA inoculation significantly increased ankle diameter and paw volume. Rats administered adjuvant alone showed significant increase in the tissue lipid peroxidation markers and a significant decrease in the activities of tissue enzymic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase. Moreover, the increase in the level of inflammatory markers such as NF-κB, TGF-β, TNF-α, IL-1β, IL-6 and CRP and decrease in the level of IL-10 in the serum and joint was observed in CFA inoculated rats. These CFA-induced arthritic changes, cytokine profile, and oxidative stress markers were significantly reversed by zingerone (25mg/kg body weight) supplementation in both the models when compared to arthritic control group. Thus, zingerone, through its antioxidant and anti-inflammatory effects, may be useful in the prevention of onset and severity of arthritis.

Keywords Arthritis, CFA, Zingerone, inflammatory markers, antioxidants

Signature of Student Signature of Major Advisor

Dated:_____ Dated:_____

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I bow my head before Almighty, the omnipotent, the omnipresent, the merciful, the most gracious, the compassionate, which is the entire and only source of every knowledge and wisdom endowed to mankind and who blessed me with wisdom, courage, kind parents, supportive siblings, patient family, gracious teachers, understanding friends and bestowed me with the ability to do this work. It is the blessing of Almighty (Allah) which enabled me to achieve this goal.

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Dr. Nazirah Bashir

Place : Shuhama
Dated :

CONTENTS

LIST OF TABLES

Abbreviations

A	Absorbance
@	At the rate of
b.wt.	Body weight
CAT	Catalase
CFE	Cell Free Extract
^0C	Degree Celsius
DH_2O	Distilled water
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbent Assay
Fig.	Figure
FCA	Freund's Complete Adjuvant
g	Gram
GPx	Glutathione peroxidase
GSH	Glutathione
GR	Glutathione Reductase
IU	International Unit
IL	Interleukin
h	Hour (s)
KCL	Potassium Chloride
kDa	Kilo daltons
Kg	Kilogram (s)
L	Litres
LDL	Low density Lipoproteins
LPO	Lipid peroxidation
µg	Microgram (s)
µl	Microlitre
µM	Micromolar

M	Molar
m	Meter
mg	Milligram
ml	Milliliter
mm	Millimeter
mM	Millimoles
mm^2	Square millimeter
mn	Minute (s)
MDA	Malondialdehyde
N	Normal
ng	Nanogram
/	Per
%	Percent
PMS	Post Mitochondrial Supernatant
rpm	Revolutions per minute
SOD	Superoxide dismutase
TP	Total Protein
NFκB	Nuclear Transcription Factor- Beta
TNF-α	Tumor necrosis factor- alpha
TGF-β	Transforming Growth factor -beta
Xg	Times gravity
U	Unit(s)
UV	Ultra Violet
V	Volt
w/v	Weight/Volume

Chapter-1

INTRODUCTION

Arthritis a multi-factorial inflammatory disease affects roughly 0.5-1% of the adult population worldwide (Kazantseva *et al*., 2012; Kurko *et al*., 2013).The disease represents a rising public health problem owing to its debilitating and chronic symptomatology (Pedersen *et al*., 2009; Soubier *et al*., 2010; Cross *et al*., 2014). The disease is often progressive and results in pain, stiffness, and swelling of joints and in later stages, deformity and ankylosis develop leading to substantial loss of functioning and mobility. In animals, arthritis is a major manifestation of a complication of mycoplasma disease and is caused by *Mycoplasma agalactie* in sheep and goats (Cottew, 1970), *M. mycoides* in cattle (Piercy and Bingley,1972), *M. hyorhinis* in swine (Mcduffie and Imura,1970; Ross and Duncan,1970), *M. arthritidis* in rats and mice (Piercy,1970) and *M. gallisepticum* and *M. synoviae* in chickens and turkeys (Kerr and Olsonn,1970). The chief pathological features of the disease include the formation of an inflammatory erosive synovitis that ultimately leads to destruction of cartilage, bone and soft tissues resulting in loss of joint function. Although joints are the main target of disease process in arthritis, the disease is usually classified as a non organ specific autoimmune one, because of the occurrence of extra articular features, such as subcutaneous nodules, vasculitis and pulmonary fibrosis (Williams, 2007).

The onset of the disease comprises the recognition of self-protein epitopes by auto-reactive T and B cell clones, inducing a strong inflammation within the joints (Scott *et al*., 2010). This process is mainly mediated by the activation of Th1 and Th17 cells that are responsible for the secretion of cytokines. In combination, these cytokines prompt the activation of innate immune cells, such as neutrophils, macrophages and dendritic cells, leading to bone and articular degradation by osteoclasts, reduced cartilage repair and pain (McInnes and Schett,

2007; Hueber *et al*.,2010; Zhang *et al*.,2011; Yang and Karin, 2014). Moreover, anti-citrullinated protein antibodies (ACPA) of both the IgG and IgA classes are produced in the mucosal tissues, such as lungs and at the site of inflammation. Levels of synthetic cyclic citrullinated peptides (CCP) in serum and synovial fluid are even markers of arthritis onset, progression and severity of the disease (Kroot *et al*., 2000; Snir *et al*., 2010).

A group of inflammatory cytokines participate in the pathogenesis of arthritis and are amongst key factors responsible for loss of metabolic homeostasis of tissues forming joints by promoting catabolic destructive processes. They play a key role in the pathogenesis of arthritis through a cascade of biochemical alterations of the majority of cells in the joint and intracellular pathways of signal transduction for the production of cytokines and other inflammatory compounds and enzymes. Tumor necrosis factor alpha (TNF-α), together with interleukin one beta (IL-1β), is a key inflammatory cytokine involved in pathophysiological processes occurring in the course of arthritis. TNF-α is secreted by cells in the joint responsible for the synthesize of IL-1β, and increased levels of both the inflammatory cytokines is observed in synovial fluid, synovial membrane, cartilage and sub- chondral bone layer (Farahat *et al*., 1993; Sohn *et al*., 2012). In the cells of joint, IL-1β induces its own secretion in an autocrine manner and stimulates the synthesis of other cytokines such as TNF-α, interleukin six (IL-6), interleukin eight (IL-8), and CCL5 chemokine (Pulsatelli *et al*., 1999; Aigner *et al*., 2005). IL-1β inhibits the signal pathway of the R-SMAD proteins (receptor-regulated SMAD), responsible for the activation of transcription factors associated with transforming growth factor beta (TGF-β) (Bauge *et al*., 2008).

During the course of the disease, IL-1β stimulates the production of reactive oxygen species (ROS), with the formation of peroxides and hydroxylated radicals and decreased expression of oxidative enzymes. These processes accelerate further damage of articular cartilage in the joint affected by the disease (Afonso *et al*., 2007). The effect of TNF-α in most cases coincides with the action

of IL-1β, and during the course of arthritis there is a marked synergism between the two cytokines (Henderson and Pettipher, 1989). This effect is the result of activation of the same group of intracellular signaling pathways, which in turn triggers similar effects that increase the inflammation and catabolism in joint tissues (Roman-Blas and Jimenez, 2006; Marcu *et al*., 2010). So there occurs a complex interplay between ROS and inflammatory mediators.

A panel of drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease modifying anti- rheumatoid drugs (DMARDs), biologicals such as TNF-α and IL antagonists and JANUS kinase inhibitors (JKIs) have been used to relieve pain and reduce immunological reaction mediated inflammation and joint damage (He *et al*., 2013; Singh *et al*., 2016). A step wise approach starting with NSAIDs followed by glucocorticoids and DMARDs, either alone or in combinations is being made in the current management of arthritis (Yamanaka *et al*., 2016). However, in response to recent evidence, NSAIDs have lost their prominent role as first-line treatment because of concerns about their limited effectiveness, inability to alter the long-term course of disease and the associated side effects like gastrointestinal or cardiac toxicity (Schaffer *et al*., 2006; Scott *et al*., 2007). The adverse effects of long term glucocorticoid treatment are common and include immune suppression, osteoporosis, and metabolic disorders (Baschant *et al*., 2012;2013). In recent years, biological agents especially tumor necrosis factor (TNF) inhibitors are widely used in the treatment of disease (Feldmann and Maini, 2008). However, the increased infection rate and high costs restrict the prescription of these biological agents (Cohen and keystone, 2015; Atzeni *et al*., 2016).

Due to the limitations of above therapeutic approaches, there is need for the development of biomolecules derived from plants or natural sources devoid of adverse effects. India, “the Botanical garden of the world” is the largest producer of medicinal plants. The medicinal plants and herbs are playing an important role in the health and vitality of human beings as well as animals. As per WHO estimates, about three quarters of the world’s population currently use herbs and

other medicinal plants to cure various diseases, including arthritis. Hence several phytomedicines (medicinal plants or herbal drugs) are now used for the prevention and treatment of arthritis. Herbal drugs have gained importance and popularity because of their safety, efficacy and cost effectiveness. The Indian traditional medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Despite enormous advances in modern medicine, there are no completely effective drugs that offer complete protection from the disease. In recent decades, many herbs and natural compounds have been receiving increasing public interest as complementary and alternative medicine (CAM) (Ahmed *et al*., 2005) and both clinical and basic research laboratories have focused their attention to find out remedial measures out of these native medicines. Thus, it is necessary to identify pharmaceutical alternatives for the treatment of diseases, with the aim of these alternatives being more effective and less toxic. Plants (or supplements derived from the plants) that have received attention as being useful for chronic inflammation include limonene, naringenin, emodin, resveratrol, ginger (Wohlmuth *et al*., 2005).

Ginger (*Zingiberofficinale Roscoe*; family Zingiberaceae) is a monocotyledonous, which is native to India or Southeast Asia, from where it was introduced to other parts of the world

(Ravindran ,1994). Both fresh and dried ginger rhizomes are used worldwide as a food additive and a spice as well as a phytomedicine since ancient times (Ghayur and Gilani, 2005).

Z. officinale rhizome constituents vary depending upon the area of origin as well as on rhizome conditions. The rhizome constituents include fat, minerals, proteins, lipids, fibers, fatty acids, carbohydrates, lecithins, protease, calcium, phosphorous, potassium along with many vitamins like riboflavin, thiamine, vitamin C and niacin (Ibrahim *et al*.,2010).The health-promoting perspective of ginger is often attributed to its rich phytochemistry. Phytochemical studies showed the presence of pungent principles, such as gingerol, shogaol, zingerone,

and paradol are the main aroma defining component is zingiberol (Varma *et al*., 1962).

Several studies have indicated that compounds found in ginger are effective in relief of symptoms from chronic inflammatory diseases (Grzanna *et al*., 2005). Zingerone belongs to phenolic alkanone group, and have a wide range of pharmacological properties including antioxidant (Rajan *et al.,* 2013), anti-inflammatory (Kim *et al.,* 2010), anticancer (Kumar *et al.,* 2013), antimicrobial (Ahmad *et al*., 2015) and antidiabetic activity (Ahmad *et al*., 2018).

In view of the above-mentioned immense properties of the zingerone the present study was designed with the following objectives:

Objectives

1. To investigate the effect of zingerone on the symptomatology of Freund's Complete Adjuvant induced arthritis
2. To investigate the effect of zingerone on various inflammatory markers in Freund's Complete Adjuvant induced arthritis
3. To investigate the anti-oxidant effect of zingerone in Freund's Complete Adjuvant induced arthritis

Chapter-2

REVIEW OF LITERATURE

Inflammatory cytokines (e.g. tumor necrosis factor and interleukin-1β) and pro-inflammatory enzymes that mediate the production of prostaglandins (e.g. cyclooxygenase-2) and leukotrienes (e.g. lipooxygenase), together with the expression of adhesion molecules and matrix metalloproteinases, and hyperproliferation of synovial fibroblasts. All of these factors are regulated by the activation of the transcription factor nuclear factor-κB. Thus, agents that suppress the expression of tumour necrosis factor-α, interleukin-1β, cyclooxygenase-2, lipooxygenase, matrix metalloproteinases or adhesion molecules, or suppress the activation of NF-κB, all have potential for the treatment of arthritis.

Arthritis is a form of joint disorder that involves inflammation in one or more joints with over 100 different forms (Son *et al*., 2007). Although there are more than 100 different kinds of arthritis, the three most common forms worldwide are gout, osteoarthritis (OA) and rheumatoid arthritis (RA). Gout occurs in response to the presence of monosodium urate (MSU) crystals in joints, bones and soft tissues, and is usually treated by non-steroidal anti-inflammatory drugs (NSAIDs), oral or intravenous colchicines, and oral, intravenous or intrarticular glucocorticoids. All can abort acute attacks, but they also may have severe side effects (Sangha,2000).

Osteoarthritis is a type of age-related joint disease characterized by the breakdown of joint cartilage and underlying bone in the joints induced by a combination of genetic, metabolic, biochemical and biomechanical factors (Rahmati *et al.,*2016; Poulet and Staines,2016).With an increasing aging population, the prevalence of osteoarthritis is expected to increase and become the leading cause of disability in future decades(Xue *et al*.,2015). The degree of articular inflammation is usually related to disease progression, suggesting articular injury as a factor in this disease (Chen *et al*.,2014). Recent studies demonstrate that chondrocytes can release and respond to many different cytokines, which are important active players during disease progress (Lepetsos and Papavassiliou, 2016). Proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, are elevated in the synovium and synovial fluid in osteoarthritis and play a key role in the pathology of osteoarthritis (Zhang *et al*., 2017). OA is normally treated with analgesics

such as acetaminophen and opioids, NSAIDs, and intraarticular therapies such as glucocorticoids and hyaluronans.

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and infiltration and proliferation of synovial tissue of the joints (Majithia and Geraci, 2007). About 1% of the world's population is affected by RA, with women three times more often than men (Kvien, 2004). Although the pathophysiological basis of RA has not yet been fully understood, immunological events associated with genetic factors are thought to be the basis of RA development (Gomes *et al*., 2011). The complex interaction of different immune modulators (cytokines and effector cells) is responsible for joint damage starting at the synovial membrane and involving most of the articular structures (Smolen and Steiner, 2003). As a result of the reaction against antigens in the joint, activated inflammatory cells such as macrophages, T-cells, and B-cells cause activation or release of degenerative enzymes of other cells, TNF-α, antibodies and ROS. Thus, they increase periarticular tissue degeneration and joint deformation, leading to disease progression (Neergheen-Bhujun *et al*., 2014; Sarban *et al*., 2005). The goals of management of patients with RA are to control pain and swelling, delay disease progression, minimize disability, and improve quality of life. For pain control and swelling, treatment includes analgesics such as acetaminophen and opioids, NSAIDs, and intra-articular therapies such as glucocorticoids. In addition, diseases modifying anti-rheumatic drugs (DMARDs) are used to modify the clinical and radiological course of RA. Examples include methotrexate (MTX), sulfasalazine, leflunomide, hydroxychloroquine and newer therapies such as anti-tumour necrosis factor (TNF-α) therapy (etanercept, infliximab and adalimumab), anti-CD20 therapy (rituximab) and abatacept. However, all of these agents are associated with numerous side effects.

Much progress has been made in recent years towards the identification of mediators that contribute to the pathogenesis of arthritis, and a number of studies have pointed to a pivotal role of various immune markers in the disease process. Indeed, the success of biological inhibitors of immune markers in the clinic is a testament to the pathological significance of these immune markers in arthritis. However, there is still lack of knowledge of underlying causes of the diseases and it is for this reason, together with

the need for more durable remedies, that animal models of arthritis continue to be studied. Animal models of arthritis are used in a wide variety of different studies, including preclinical testing of novel therapies, analyzing mechanisms of drug action, identifying both pro- and anti-inflammatory mediators, analyzing genetic susceptibility factors, and in the search for markers of disease progression.

Adjuvant induced arthritis

Adjuvant arthritis was the first model of arthritis to be described and can be induced in rats by a single injection of Freund's adjuvant, containing *Mycobacterium tuberculosis* (Pearson,1956). Clinical arthritis starts at around 10-14 days after injection and generally subsides after 1 month. The chief pathological features of adjuvant induced arthritis include oedema, infiltration into the joint of mononuclear and polymorphonuclear cells, pannus formation, periostitis, and erosion of cartilage and bone. Although an association between immuntity to 65-kDa heat shock proteins and the induction of adjuvant arthritis has been suspected (van Eden *et al*., 1988), no single mycobacterial immnogen has been shown to be responsible for the arthritogenic response in the model (Holmgahl *et al*., 1992). Rather, the induction of adjuvant arthritis has been attributed to a mycobacterial cell wall component, muramyl dipeptide, which is immunostimulatory but does not evoke a specific immune response (Kohansi *et al*., 1982). In addition, a number of adjuvants which lack immunogenic properties have been shown to induce arthritis in susceptible strains of rats, including avridine(Chang *et al*., 1980), incomplete Freund's adjuvant and pristine (Holmdahl *et al*.,1992). The mechanism of arthritis induction following immunization with adjuvants is unknown, but one possibility is that following immunization there is an increase in the activity of antigen presenting cells (APCs) (Waren *et al*., 1986), leading to the presentation to autoreactive T cells of a hitherto unrecognized or sequestered endogenous antigen. The possibility that arthritis could also be triggered by exposure to environmental factors with adjuvant like activity has been highlighted by studies in which it was found that arthritis could be induced in rats by percutaneous exposure of adjuvant oils (Kleinau *et al*., 1994), or even a mineral oil-containing cosmetic product (Sverdrup *et al*., 1998).

Costa *et al*. (1981) demonstrated adjuvant-induced arthritis as a suitable model for the study of chronic pain and the effects of morphine and naloxone on it. They concluded that among the behavioral elements analysed in adjuvant-induced arthritic rats,

only scratching accomplishes the criteria of chronic pain behavior, it is significantly and chronically increased in the arthritic rats, it is reversed (depressed) by morphine, this effect being blocked by naloxone.

Doughty *et al*. (1991) used Rat adjuvant arthritis (AA) as a model to evaluate several blood markers as possible predictive indicators of drug efficacy. By analyzing the various blood markers: hyaluronate by ELISA, prostaglandin E2 by RIA, ESR by micro-dispette, total PMN by Technicon H-l, and albumin by BCG dye, the relative rank order of drug efficacy (indomethacin, diclofenac sodium, Levamisole and prinomide) did not differ using the change in paw circumference or blood markers .The study concluded that, these blood markers provide additional information for the statistical evaluation of drugs in rat adjuvant arthritis.

Kawahito *et al*. (2000) investigated the synovial tissue localized expression of Peroxisome proliferator–activated receptor (PPAR-γ) in patients with rheumatoid arthritis (RA). The study revealed markedly enhanced expression of PPAR-γ in macrophages, as well as modestly enhanced expression in the synovial lining layer, fibroblasts, and endothelial cells. Activation of the PPAR-γ by 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) and the synthetic PPAR-γ ligand (troglitazone) induced RA synoviocyte apoptosis in vitro. Moreover, intraperitoneal administration of these PPAR-γ ligands ameliorated adjuvant-induced arthritis with suppression of pannus formation and mononuclear cell infiltration in female Lewis rats. Antiinflammatory effects of 15d-PGJ2 were more potent than troglitazone.

Bao *et al*. (2001) studied the potential role of cytokines in the spinal cord of AIA and changes of glial and cytokine expression (IL-1α, IL-6, TNF-α and IFN-α) in the spinal cord of AIA rats. The study indicated that macroglia and MHC class II immunostaining were enhanced. Using in situ hybridization and immunohistochemical methods, both mRNA and protein levels of IL-1α, IL-6 and TNF-α were significantly increased in the spinal cord of arthritic rats. Increased cytokine expression was presented in the reactive astrocytes and microglia.

Abd Elhalem *et al*. (2018) reported the therapeutic paracrine action of bone marrow mesenchymal stem cells (MSCs) on the IL-9 level in adjuvant-induced arthritis (AIA) and the enhancement effect of Hesperidin (Hsd) on transplanted MSCs. Results of this study confirmed that MSCs decreased Antinuclear autoantibodies, tumour necrosis

factor-alpha (TNF-α), IL-9, IL-4, interferon gamma (IFN-γ), and transforming growth factor-beta1 (TGF-β1), as well as malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) levels in AIA.

Natural pharmacological drugs have a boon of lacking or having minimal side effects compared to synthetic drugs. Over the millennia, products like ginger,garlic and turmeric have a history of acting as medicine, as they are known tocontain biologically active compounds. They are being used as the most commonflavouring agents in food. Modern knowledge of chemistry has bought in an era for studying and using these natural products, Thus, modern research like genomics, proteomics, metabolomics, synthetic and semisynthetic drug synthesisshould go hand-in-hand with the knowledge of natural drugs and compounds.

Gokhale *et al.*(2002) revealed that the ethanolic extracts of *S. lappa, A. speciosa* and *A. aspera* possess anti-inflammatory and anti-arthritic activity and support the rationalebehind the traditional use of these plants in inflammatiory conditions.The ethanolic extract of these plants not only significantly and dose-dependently inhibited the edema, also prevented recruitment of cells into the peritoneal cavity and strongly inhibited TNF-α release from LPS-stimulated murine macrophage cell line and suppressed the proliferation of lymphocytes.

Barsante *et al.* (2005) explored the effects of atorvastatin in a model of adjuvant-induced arthritis in rat. Oral treatment with atorvastatin after arthritis induction caused inhibition of the increase in paw volume and markedly ameliorated the histopathological findings of joints by an effective blockade of neutrophil influx, as assessed by the tissue myeloperoxidase levels. The concentrations of the cytokine's interleukin-1β, interleukin-6 and tumor necrosis factor-α and the chemokines, CCL5 and CCL2 were significantly decreased in arthritic rats treated with atorvastatin. The drug also prevented the hyper nociception observed in the inflamed joints.

Rajendran and Krishnakumar (2010) investigated the anti-arthritic activity of ethanol extract of *Premna serratifolia* Linn., wood in Freund's adjuvant induced arthritis model. Loss in body weight during arthritis condition was corrected on treatment with ethanol extract and standard drug, indomethacin. Biochemical parameters such as hemoglobin content, total WBC, RBC, erythrocyte and sedimentation rate were also estimated. The ethanol extract at the dose of 300 mg/kg body weight inhibited the rat paw

edema by 68.32% which is comparable with standard drug indomethacin 74.87% inhibition of rat paw edema after 21 days.The observed antiarthritic activity may be due to the presence of phytoconstituents such as irridiod glycosides, alkaloids, phenolic compounds and flavonoids.

Ramadan *et al*. (2011) compared the anti-inflammatory and anti-oxidant activity of *Curcumalonga* (Turmeric) Versus *Zingiberofficinale* (Ginger) plants in rat adjuvant-induced arthritis (AIA). Both plants significantly suppressed (but with different degrees) the incidence and severity of arthritis by increasing/ decreasing the production of anti-inflammatory/pro-inflammatory cytokines, respectively, and activating the anti-oxidant defence system. The anti-arthritic activity of turmeric exceeded that of ginger and indomethacin (a non-steroidal anti-inflammatory drug), especially when the treatment started from the day of arthritis induction.

Monica *et al*. (2010) evaluated and compared antioxidant capacity and radical scavenging activity of naringin and its aglycone by different *in vitro* assays. The results showed that naringenin exhibited higher antioxidant capacity and hydroxyl and superoxide radical scavenger efficiency than naringin. Additionally, naringenin showed a greater effectiveness in the protection against oxidative damage to lipids in a dose-dependent manner. Both flavanones were equally effective in reducing DNA damage.

Arora *et al.* (2014) reported the protective potential of Curcumin loaded solid lipid nanoparticles (C-SLNs) in ameliorating complete Freund's adjuvant (CFA)-induced arthritis in rats through attenuation of oxido-inflammatory and immunomodulatory cascade.C-SLN administration significantly and dose dependently ameliorated various symptoms of arthritis in rats, improved biochemical markers and preserved radiological alterations in joints of arthritic rats. Further, the results emphasized that SLNs are a novel approach to deliver curcumin into the inflamed joints and improve its biopharmaceutical performance.

Bhardwaj *et al.* (2016) studied the anti-arthritic activity of ethanol and aqueous extract of root of *Ficusbenghalensis* on Freund's adjuvant induced arthritis in rats. The results indicated that both the extracts protect the rats against primary and secondary arthritic lesions, body weight changes and haematological perturbations induced by FCA. The observations showed that ethanol extract show highly inhibition of paw edema in rats.

Funk *et al.* (2016) assessed anti-inflammatory effects of ginger's other secondary metabolites, the essential oils (GEO), which contain terpenes with reported phytoestrogenic activity, in female Lewis rats with streptococcal cell wall (SCW)-induced arthritis. GEO prevented chronic joint inflammation, but had no effect on the initial acute phase of joint swelling and granuloma formation at sites of SCW deposition in liver.

Ruckmani *et al*.(2017) evaluated the effects of two doses (400 and 800 mg/kg) of ethanolic extract of *Sesamumindicum* seeds in Freund's complete adjuvant induced arthritis in rats in comparison with diclofenac and methotrexate by the changes produced in body weight, body temperature, paw volume and spontaneous activity, hemoglobin, erythrocyte sedimentation rate, total white blood cells, red blood cells, Interleukin-6 and Tumor necrosis factor-α as well as joint changes in X-ray and histological changes in joint tissue.The effect of ethanolic extract of *Sesamumindicum* was found to be equivalent to that of methotrexate and greater than diclofenac.

Gohil *et al*. (2017) reported the anti-arthritic activity of cell wall content of *Lactobacillus plantarum* in complete Freund's adjuvant (CFA)-induced arthritis in rats. Cell wall content of *L. plantarum* treated animals showed improvement in all the parameters viz., change in body weight, paw volume and arthritic index, joint stiffness, gait test, mobility test, erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP) level, serum rheumatoid factor (RF), and serum TNF-α as compared to that in CFA-treated control animals and hence exert anti-arthritic activity.

Zhang *et al.* (2017) studied the effects and mechanisms of Rg1, the active ingredient of *Panax ginseng* on adjuvant-induced arthritis (AIA) in rats with its anti-inflammatory effects by up-regulating PPAR-γ and subsequent inhibition of NF-κB signal pathway. The results showed that Rg1 significantly alleviated joint swelling and injuries and reduced the level of TNF-α and IL-6, increased PPAR-γ protein expression, inhibited IκBα phosphorylation and NF-κB nuclear translocation in the inflammatory joints of AIA rats and RAW264.7 cells stimulated by lipopolysaccharide (LPS).

Deepaand Snehal (2017) evaluated the anti-arthritic activity of hecogenin in Complete Freund's adjuvant-induced arthritis in rats. Treatment of rats with hecogenin and its combination elicited significant reduction in paw edema, arthritic score and joint diameter and also inhibited joint destruction in histopathological and radiological

analyzes of ankle joint. The anti-arthritic activity was also confirmed with the change in biochemical parameters and myeloperoxidase assay.

Cai *et al.* (2017) investigated the potential therapeutic effect of acetazolamide (AZ, an AQP1 inhibitor) on rat adjuvant-induced arthritis (AIA) and explore its related mechanisms.AZ treatment inhibited secondary hind paw swelling and arthritis index, reduced serum levels of TNF-α and IL-1β, and ameliorated pathological changes of ankle joint in AIA rats. AZ increasedproteoglycans production and mRNA levels of COII and aggrecan in cartilage tissues. Moreover, AZ decreased AQP1 protein level and suppressed the activation of NF-κB pathway in synovium by inhibiting the degradation and phosphorylation of IκBα and reducing p-NF-κB p65 protein level.

Pan *et al*. (2018) reported thatAcamprosate Protects Against Adjuvant-Induced Arthritis in Rats via Blocking the ERK/MAPK and NF-κB Signaling Pathway.They found that acamprosate significantly suppressed paw swelling and the arthritis index in AIA rats. Moreover, acamprosate also significantly suppressed the production of TNF-α, IL-1β, and IL-6 in serum, which is elevated by AIA induction. Finally, acamprosate inhibited p-c-Raf and p-ERK1/2 and NF-κB activation after AIA treatment.

Wang *et al.* (2018) reported sorafenib exerts anti-arthritic effects in Adjuvant-Induced Arthritic rats and therefore has potential in RA treatment. The data revealed that sorafenib administration led to significant body weight gain in AA rats but suppressed paw swelling, synovial hyperplasia, and inflammatory infiltration. Furthermore, it decreased TNF-α, IL-1β, and IL-17 serum levels and up regulated IL-10. Microvascular density (MVD) and the expression of vascular endothelial growth factor receptor 2 (VEGFR-2) and fibroblast growth factor receptor 1 (FGFR-1) in synovial tissues were significantly reduced.

Mbiantcha *et al.* (2018) illustrated the anti-inflammatory and anti-arthritis potential of methanol extract of *Boswelliadalzielii*(BDME). BDME revealed a significant anti-inflammatory effect by preventing the development of edema caused by carrageenan, arachidonic acid, histamine, serotonin, prostaglandin and bradykinin. For anti-arthritic properties of BDME,the results showed a significant reduction of the joint diameter and a decrease in pain in the treated animals.. Furthermore, BDME exhibited significant inhibition of extracellular and intracellular ROS production and displayed significant

inhibitory activity on T-cell proliferation as well as a reduced production of TNF-α and IL-1β.

Lee (2018) examined the relationship between neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), mean platelet volume (MPV), and rheumatoid arthritis (RA), to establish a correlation among the NLR, PLR, and MPV and RA activity.Meta-analysis showed that the NLR and PLR were significantly higher in the RA patients and positively but weakly correlated with the RA activity.

Shrijani *et al.* (2018) investigated the anti-arthritic activity of the seed oil of *Vateriaindica*against complete Freund's adjuvant (CFA) induced arthritis in rats.Physical parameters such as paw volume, diameter and body weight, serum parameters such as SGOT, SGPT, ALP and RF showed dose dependent significant decrease when compared to arthritic control group. All treated animals showed significant decrease in serum rheumatoid factor.

Zuo *et al.* (2018) investigated effects of α-Mangostin (MG) on adjuvant-induced arthritis (AA) in rats and found that MG possessed superior anti-inflammatory effects in vivo, suggested by attenuated paw swelling, reduced inflammatory cells infiltration and decreased the secretion of TNF-α and IL-1β in serum and Inhibited NF-κB. MG inhibited fibrous hyperplasia, synovial angiogenesis, and cartilage and bone degradation in AA rats. Although MG exerted little effects on $CD4^+$ population, it greatly decreased IFN-γ positive cells and promoted expression of FOXP3 in immune organs, indicating restoration of Th1/Treg cells ratio and recovery of immune homeostasis in vivo.

Pal *et al.* (2018) reported the protective effect of mangiferin and its interaction with low dose of nitric oxide (NO) modulators in complete Freund's adjuvant (CFA) inoculated rats. The CFA-induced arthritic resulted in the changes in arthritic index, ankle diameter, paw volume, cytokine profile (serum TNF-α, IL-6, IL-1β, serum Th1 (IFN-γ) , Th2 (IL-4) and synovial TNF-α levels), and oxidative stress markers were significantly reversed by mangiferin treatment alone and in combination with l-arginine and l-NAME nitric oxide modulators .

Geferson *et al.* (2018) reported that rosemary aqueous extracts possess efficient anti-inflammatory agents and suggested that they are able to attenuate the oxidative stress inherent to arthritis. Administration of the rosemary extract (RE) to arthritic rats diminished oxidative damage (e.g., less carbonylated proteins), improved the oxidative

state (e.g., less reactive oxygen species) and also increased the antioxidant capacity by increasing the GSH level and the GSH/GSSG ratio there by normalizing the activity of several antioxidant enzymes. Treatment or arthritic rats with the extract also diminished the paw edema; the number of leukocytes recruited in the femoro-tibial joint cavities and the weight of the lymph nodes and delayed the appearance of secondary lesions.

Saleem *et al.* (2019) reported the anti-arthritic potential of *M. rivae* extracts in chronic polyarthritis model. Treatment of adjuvant-induced arthritic rats with methanolic and aqueous extracts of *M. rivae* significantly restored arthritic index, change in the body weight and immune organ weight, and the histopathological indices. Both extracts significantly reduced the serum concentration of rheumatoid factor, C-reactive protein, PGE2, and TNF-α in arthritic rat. These extract down-regulated the COX-2, PGE2, IL-1β, IL-6, NF-κB, and TNF-α, and up-regulated the mRNA expression of I-κB, IL-4 and IL-10. Both extracts increased the activities of CAT and SOD while reducing the formation of MDA in a dose- dependent manner in the liver.

Ginger is also one of the most commonly used condiment and a natural drug invogue. It is a traditional medicine, having some active Ingredients used for the treatment of numerous diseases. Several studies have reported the protectiveeffects of ginger on different organs and tissues. Nwaopara *et al*. (2008) and Shatiand Elsaid(2009) demonstrated that ginger showed significant amelioration onoxidative stress induced by alcohol abuse and changes in liver, kidney and brain of rats tissue. Ramudu *et al*. (2011) showed that ginger protected the liver tissuefrom Streptozotocin induced oxidative damage. In addition, treatment with 1 % ofdietary ginger for 4 weeks to rats improved antioxidant status, which may haveprotective role in hepatic tissue (Mallikarjuna *et al*., 2008). Moreover, treatmentby ginger showed marked regeneration and improvement in the hepatic tissues ofalbino rats and significant decrease in serum levels of liver enzymes comparedwith untreated diabetic group (Abd-El Aty and Morgan,2011). Bhandari *et al*.(2005) reported that ginger could protect the liver and pancreatic tissues fromlipid peroxidation on Streptozotocin (STZ) diabetic rats. Furthermore, Chakraborty *et al.* (2012) revealed that ginger has been shown to modulate insulin release in pancreatic B-cell and reported that gingerol which is an active component of ginger showed aprotective effects on pancreatic B-cell. Jafri *et al.* (2010) has reported thatginger appeared to show the hypoglycemic effect in alloxan induced diabetic rats.

During recent research on ginger, various ingredients like zingerone,shagol and paragol have been obtained from it. Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) is a nontoxic and inexpensive compound with variedpharmacological activities. It is the least pungent component of *Zingeber officinale*. Zingerone is absent in fresh ginger but cooking or heating transtormsgingerol to zingerone. Zingerone closely related to vanillin from vanilla andeugenol from clove. Zingerone has potent anti-inflammatory, anti-diabetic, anti-lipolytic, anti-diarrhoeic, anti-spasmodic,etc. properties. Besides, it displays the property of enhancing growth and immune- stimulation.

Zingerone is present in a significant amount of about 9.25% in ginger. It is a member of Methoxyphenol family and its related derivatives. They have a basic phenolic ring with a methoxy group attached to benzene ring. Use of high profileliquid chromatography has shown that the contents of 6-gingerol, 8-gingerol and10-gingerol; are usually low in fresh ginger while, on drying and roasting the amount of zingerone increases significantly.

The first evidence suggestive of antioxidant properties exhibited by zingerone is that it has ability to degrade free radicals generated by radiolysis of various food products. The observation that zingerone minimizes oxidation of lipids, undoubtedly signifies its role as an antioxidant. It was supported by the fact that zingerone suppresses ferric ascorbate induced lipid peroxidation in rat brain(Rajkumar and Rao, 1994). Zingerone has been reported to protect im vitro DNA against stannous chloride induced ROS oxidative damage(Rajan *et al.,*2013). Zingerone provides direct adaptogenic effect by preventing oxidative stress on smooth muscles of intestine (Banji *et al*., 2014). Further, zingerone administration was found to reduce the mitochondrial injury, peroxidation oflipids and down regulation of some pro-apoptic proteins like Bax, Apaf-1 andCaspase 3-9. These findings lead to a conclusion that zingerone is a potent antioxidant.

Vinothkumar *et al*.(2014) have shown that zingerone has anticancer potential. It has been proved that supplementation with zingerone in DMH (Dimethyl hydrazine)treated rats lead to a significant decrease in tumor incidence and aberrant crypt foci formation with simultaneous modulation in the levels of tissue lipid peroxidation and antioxidant status.

Chen *et al.*(2007) have shown that zingerone has ability to inhibit enterotoxins of various pathotypes of *E.coli* induced fluid secretion in the ileum of mice andinhibits colonic motility not only in vitro but also in-vivo in rats. Since abnormal facilitation of

gastrointestinal motility and excessive fluid secretion of gastrointestinal tract causes diarrhoea, zingerone is likely the active principle responsible for the anti-diarrhoeal activity of ginger. Some natural pungent compounds such as capsaicin activate a non-selective cation channel termed transient potential vanelloid-1(TRPV1). Zingerone also has been demonstrated to evoke opening of TRPVI. Zingerone a pungent analogue of zingerol exerts an inhibitory effect on colonic motility by TRPV1 blockade(Iwami *et al*.,2011). Zingerone is responsible for anti-diarrhoeal activity of gingerand it modifies bacterial as well as host cell metabolism.

Kim *et al.*(2010) studied the modulation of age-related NF-kappa B activation by dietary Zingerone via MAPK pathway. Zingerone treatment suppressed gene activation of pro-inflammatory enzymes, COX-2 and iNOS, which were upregulated with aging through NF-kappaB activation and IKK/MAPK Signaling pathway. These experiments strongly indicate that zingerone treatment exerts a beneficial efficacy by suppressing both oxidative stress and age-related inflammation through the modulation of several key pro-inflammatory genes and transcription factors. Thus, the significance of these findings is that the zingerone treatment may provide some preventive measures against chronic inflammatory conditions that underlie many age-related inflammatory diseases, such as metabolic syndrome, cardiovascular disease,dementia, arthritis, diabetes, osteoporosis and cancers.

Rao *et al.*(2011) studied the protective effect of zingerone against radiation induced genetic damage and apoptosis in human lymphocytes. This study revealed that protective effect of zingerone is attributed to scavenging of radiation induced free radicals and also by the inhibition of radiation induced oxidative stress.

Han *et al.*(2011) studied the effects of zingerone on fat storage in ovariectomised rats. This study revealed that zingerone significantly reduced bodyweight and the final parametrial adipose tissue weight in ovaricctomized rats. Blood glucose meeasured after oral administration of glucose were lower in zingerone treated ovariectomized rats. Zingerone significantly increased the norepinephrine induced lipolysis associated with the translocation of hormonesensitive lipase from cytosol to lipid droplets in adipocytes.

Chang *et al.*(2012) reported that dietary administration of zingerone enhances growth, non-specific immune response, and resistance to *Vibrio alginolyticus* in Pacific white shrimp (*Litopenaeus vannamei*) juveniles.Zingerone can be recommended as a

supplement to shrimp feed to increase growth, immunity, and disease resistance against the pathogen,*Vibrio alginolyticus* and acts as appetizer and immunostimulant in shrimp is quitepromising.

Kumar *et al.*(2013) reported that zingerone inhibits biofilm formation andimprove antibiofilm efficacy of ciprofloxacin against *Pseudomonas aeruginosa*.Multidrug resistant opportunistic pathogen *Pseudomonas aeruginosa* produces surface-associated communities called biofilms, which protect pathogen byforming a complex permeability barrier for antibiotics and immune cells. Biofilm formation contributes to persistent and chronic infections caused by *Pseudomonas aeruginosa.*Biofilm was inhibited and eradicated in presence of zingerone aloneand in combination with ciprofloxacin. Highly significant inhibition was observedwhen phytochemical and antibiotic were used as adjunct therapy. These findings prove zingerone as potential phytotherapeutic agent which in future can beemployed to formulate preventive strategies against biofilm associated infectionscaused by *Pseudomonas aeruginosa*.

Xie *et al.* (2013) reported that zingerone attenuates lipopolysaccharide-induced acute lung injury in mice. Zingerone significantly inhibited the production of LPS-induced proinflammatory cytokines in vitro and in-vivo. Pulmonary histopathologic changes, as well as alveolar hemorrhage and neutrophil infiltration were substantially suppressed in lung tissues,with evidence of reduced myeloperoxidase (MPO) activity. The lungwet-to-dryweight (W/D) ratios, as the index of pulmonary edema, were markedly decreased by zingerone pretreatment. Furthermore, the study revealed that zingerone attenuates the mitogen-activated protein kinases (MAPK) and nuclear factor-kappaB (NF-κB) signaling pathways through blocking the phosphorylation of ERK, p38/MAPK and IκBα, NF-κB/P65.

Rajan *et al.*(2013) reported that zingerone protects against stannous chloride-induced and hydrogen peroxide-induced oxidative DNA damage in-vitro. The in-vitro toxicity of stannous chloride (SnCl2) was evaluated using genomicand plasmid DNA. SnCl2 - induced degradation of genomic DNA was found tooccur at a concentration of 0.8 mM onwards.

Kumar *et al.*(2014) studied structural alterations in *Pseudomonas aeruginosa* by zingerone contribute to enhanced susceptibility to antibiotics,serum and phagocytes. The

mechanism was evaluated in terms of cell surface hydrophobicity, alginate and LPS production. TNF-alpha and MIP-2 cytokine production by mouse macrophages was also checked. Structural analysis was carried out using scanning electron microscopy (SEM) and liquid chromatography-mass spectrometry (LC-MS) analysis. Zingerone treated cellsshowed significant decrease in TNF-alpha and MIP-2 cytokine production as compared to non-treated cells. Zingerone significantly influences the surface structure of *Pseudomonas aeruginosa* which contributes towards enhanced susceptibility to antibiotics and innate immune system. Zingerone has proved tobe one such agent which can be employed as a potential anti-virulent drug candidate against *Pseudomonas aeruginosa* infections.

Kumar *et al.*(2014) studied the hepato protective effect of zingerone in lipopolysaccharide (LPS) induced mice and has been shown that it scavenges free radicals and down regulates inflammatory mediators. This study proved that zingerone is a potent anti-inflammatory molecule against LPS induced liver injury.

Banji *et al.*(2014) studied that role of zingerone regulating intestinal transit, behavioural and oxidative perturbations in irritable bowel disorder in rats.This group established that zingerone reduced colonic transit, neutophil infilitration and lipid peroxide formation. They also studied effect of zingerone onanti-oxidant enzymes.

Cheong *et al.* (2015) investigated the hepatoprotective and anti-fibrotic effects of zingerone against carbon tetrachloride (CCl4)- and dimethylnitrosamine (DMN)-induced liver injuries in rats, respectively. Oral administration of zingerone reduced CCl4-induced abnormalities in liver histology, serum alanine aminotransferase and aspartate aminotransferase levels, and liver malondialdehyde levels. Zingerone treatment attenuated CCl4-induced increases in inflammatory mediators, including tumor necrosis factor-α, interleukin-1β, cyclooxygenase-2, and inducible nitric oxide synthase mRNA levels. Western blot analysis showed that zingerone suppressed activation of nuclear factor-kappa B (NF-κB) p65 and phosphorylation of extracellular signal-regulated kinase, c-Jun NH2-terminal kinase, and p38 mitogen-activated protein kinases (MAPKs). Liver fibrosis induced by DMN) was ameliorated by administration of zingerone (10 and 20 mg/kg, orally). Zingerone treatment reduced DMN-induced elevation of hydroxyproline content and hepatic stellate cell activation. In conclusion, zingerone showed anti-oxidative and anti-inflammatory effects in CCl_4-intoxicated rats by inhibiting oxidative

stress and NF-κB activation via blockade of the activation of upstream MAPKs. Moreover, zingerone had hepatoprotective and anti-fibrotic effects against DMN-induced liver injury suggesting its usefulness in the prevention of liver inflammation and the development of hepatic fibrosis.

Rehman *et al.* (2015) investigated the protective effect of zingerone against cisplatin-induced jejunal toxicity. Zingerone ameliorated cisplatin-induced lipid peroxidation, increase in xanthine oxidase activity, glutathione depletion, decrease in antioxidant and phase-II detoxifying enzyme activities. Zingerone attenuated cisplatin-induced nuclear factor (NF-κB) activation, enhanced levels of TNF-α and Nitrite. The results showed that zingerone had not only the antioxidant effect by suppression of ROS, but also anti-inflammatory effects by suppression of NF-κB activation. In addition, zingerone treatment suppressed gene activation of pro-inflammatory cytokine, TNF-α, which was up regulated with cisplatin administration through NF-κB activation.

Hemalatha and Stanely Mainzenin(2015) evaluated the antihyperlipidaemic, antihypertrophic, and reducing effects of zingerone onisoproterenol-induced hyperlipidaemia and hypertrophy in rats. The in- vitro study revealed that treatment with zingeronedecreased serum creatine kinase and lactatedehydrogenase activities in the rats.In addition decreased levels/concentrations of serum andheart cholesterol and triglycerides,serum lipoproteins and theactivity of liver 3-hydroxy-3-methyl glutaryl-coenzyme-A-reductase were observed inisoproterenol-inducedmyocardial infarcted rats.

Mani *et al*. (2016) studied the antioxidant and anti-inflammatory role of zingerone in ethanol-induced hepatotoxicity. Ethanol alone administered rats showed significant increase in the plasma and tissue lipid peroxidation markers such as thiobarbituric acid reactive substances, lipid hydroperoxides, conjugated dienes, and a significant decrease in the activities of plasma and tissue enzymic and non-enzymic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, reduced glutathione, vitamin C, and vitamin E. Moreover, the presence of mast cells and increase in the expressions of inflammatory markers such as NF-κB, COX-2, TNF-α, and IL-6 and decrease in the expression of Nrf2 in the liver was observed in ethanol-fed rats. Supplementation with zingerone to ethanol-fed rats reversed the changes induced by ethanol in the experimental rats.

Min *et al.* (2017) reported the anti-septic effects and underlying mechanisms of zingerone against Transforming growth factor b-induced protein (TGFBIp) mediated septic responses. Zingerone effectively inhibited lipopolysaccharide-induced release of TGFBIp and suppressed TGFBIp-mediated septic responses. In addition, zingerone suppressed TGFBIp-induced sepsis lethality and pulmonary injury.

Safhi (2018) studied the protective effects of zingerone against CCl4 induced nephrotoxicity in Swiss albino mice via modulation of metabolizing enzyme, oxidative stress, inflammatory cytokines, and apoptosis. Zingerone treatment showed significant reduction in BUN, creatinine and TBARS levels and increased the antioxidant enzymes such as GSH, GPx, GR, GST, CAT, and SOD. Besides, zingerone attenuated the levels of cytokines such as IL-1β, IL-2, and TNFα and caspases 3 and 9.

Prince and Hemalatha (2017) reported the antiapoptotic effects of zingerone in isoproterenol induced myocardial infarcted rats. Zingerone treatment revealed significant preventive effects on increased heart oxidative stress markers and decreased heart antioxidant systems. Reverse transcription - polymerase chain reaction study revealed altered myocardial expressions of B-cell lymphoma gene-2, B-cell lymphoma – extra large, B-cell lymphoma-2 associated-x , Bcl - 2 associated death promoter, Fas-receptor and caspases-8,-9 and- 3 genes in myocardial infarcted rats which were significantly prevented by treatment with zingerone.

Kaygusuzoglu *et al.* (2018) studied that zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female wistar rats. The results showed that zingerone decreased the serum FSH hormone level, increased the serum E2 hormone level, and also maintained the ovarian and uterine histological architecture and integrity. In addition, zingerone increased the measured activity of antioxidant enzymes (SOD, CAT and GPx) and the GSH content, and significantly reduced MDA levels. Zingerone was able to reduce the levels of the inflammatory markers NF-κB, TNF-α, IL-1β, IL-6, COX-2 and iNOS in CP-induced ovarian and uterine damage. It also inhibited apoptosis and reduced oxidative DNA damage markers by the downregulation of caspase-3 and 8-OHdG expression coupled with an upregulated Bcl-2 level.

Cui *et al*. (2018) reported that zingerone attenuates diabetic nephropathy through inhibition of nicotinamide adenine dinucleotide phosphate oxidase 4.The study showed

that zingerone decreased the levels of serum insulin, C-peptide and glycosylated hemoglobin A1c. The levels of blood urea nitrogen (BUN), serum creatinine, urinary albumin content and albumin/creatinine ratio (ACR) were reduced by zingerone. Moreover, zingerone attenuated the pathological injuries of kidneys, reduced the surface area of Bowman's capsule, Bowman's space, glomerular tuft, and decreased the expression of collagen IV and fibronectin in kidneys in db/db mice. The high levels of triglyceride and cholesterol, and high expression of TNFα and IL-6 were decreased by zingerone. Furthermore, zingerone decreased the level of MDA and increased the content of glutathione (GSH). Zingerone significantly decreased the expression of NADPH oxidase 4 (NOX4) in vivo and in vitro. Down regulation of NOX4 was responsible for zingerone-exhibited pharmacological activities and reduction of diabetic nephropathy.

Rehman *et al.* (2018) studied the effect of zingerone in a streptozotocin/high fat diet (STZ/ HFD)-induced type 2 diabetic Wistar rat model. The treatment with zingerone markedly abrogated ROS levels, inhibited the NF-κB activation and considerably reduced level of other downstream inflammatory molecules (TNF-α, IL-6, IL-1b).Furthermore, zingerone treatment improved renal functioning by significantly decreasing the levels of kidney toxicity markers KIM-1, BUN, creatinine, and LDH and suppressed TGF-β. Collectively, these findings indicate that zingerone treatment improved renal function by anti-hyperglycaemic, anti-oxidant, and anti-inflammatory effects, suggesting the efficacy of zingerone in the treatment of diabetic nephropathy.

Ahmad *et al.* (2018) confirmed that zingerone restrained the alloxan induced oxidative stress by increasing the activity of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and reducing the per oxidative damage. The study also confirmed that zingerone suppressed the level of redox sensitive transcription factor NF-κB and downregulated other downstream inflammatory cytokines like interleukins (IL1-β, IL-2, IL-6) and tumor necrosis factor alpha (TNF-α). Moreover, the experimental findings suggested that zingerone improved the insulin levels. Taken together, the results indicated that zingerone effectively ameliorated the diabetes induced complications which provide a strong theoretical basis for zingerone to be used clinically for treatment of diabetes.

Kandemir *et al.* (2018) investigated the Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1

permeability in rat kidney. Zingerone prevented nephrotoxicity by ameliorating the histopathological alterations, oxidative stress, inflammation, apoptosis, oxidative DNA damage and renal AQP1 levels.

Kim *et al.* (2018) reported that Zingerone concentration-dependently inhibits the pacemaker potentials of ICCs through ATP-sensitive K+ channels. Zingerone-induced pacemaker potential inhibition is dependent on guanylatecyclase, PKG, NO and MAPK pathways.

Alibakhshi *et al.* (2018) studied the Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity. Administration of zingerone resulted a statistically significant reduction in lactate dehydrogenase (LDH) activity, creatinine and BUN levels of serum. Zingerone significantly decreased the tissue levels of malondialdehyde (MDA) and significantly retained the enzyme activity of catalase (CAT) and glutathione peroxidase (GPX) in kidney tissue .Zingerone did not permit the reduction of glutathione (GSH) levels in kidney tissue and by reducing the level of tumor necrosis factor (TNF)-α suppressed the inflammation produced by cisplatin. Furthermore, zingerone improved histopathological changes such as vacuolation (fat deposit), brush border loss, infiltration of leukocytes, glomerular diameters and congestion of RBCs.

Soliman *et al.*(2018) investigated the Cardio protective effect of zingerone against oxidative stress, inflammation, and apoptosis induced by cisplatin or gamma radiation in rats. Zingerone treatment significantly reduced the abnormalities in heart histology and the increase in the cardiotoxicity indices, serum lactate dehydrogenase, and creatine kinase-MB activities, as well as plasma cardiac troponin T and B-natriuretic peptide, induced by cisplatin or γ-radiation. Further, zingerone, except for superoxide dismutase, notably ameliorated the state of oxidative stress as evidenced by a significant decrease in malondialdehyde level accompanied with a significant increase in the reduced glutathione content and catalase activity. Additionally, zingerone mitigated the increase in the inflammatory markers including serum level of tumor necrosis factor-alpha, cardiac myeloperoxidase activity, and cyclooxygenase-2 protein expression. Moreover, zingerone alleviated the elevation of caspase-3 gene expression and the prominent nuclear DNA fragmentation and attenuated the decrease in mitochondrial complexes activities.

Naidu *et al.* (2018) evaluated the antioxidant potential of zingerone against High Fructose Diet Induced Non-Alcoholic Steatohepatitis in rat model.The total phenolic content and gallic acid concentration was found to be higher in the zingereone treated group indicating the antioxidant potential of zingerone.

Chapter-3

MATERIALS AND METHODS

3.1 Experimental animals

Six to eight-week-old, albino rats of either sex (160–180 g) of Wistar strain were used for the study. Animals were housed in polypropylene cages and kept at a room temperature of 25±2°C with a 12 h light/dark cycle. They were given free access to standard laboratory animal diet and water *ad libitum*. The experimental protocols involved in this study were approved by the "Institutional Animal Ethical Committee (IAEC)" vide no: AU/FVSc/VCC/1-3/19/815-16. All the animals were acclimatized for one week prior to the commencement of experiments.

3.2 Treatment regimen

To study the effect of treatment with zingerone on Complete Freund's adjuvant (CFA)-induced oxidative stress and inflammatory response in joints of Wistar rats, 24 Wistar rats were randomly divided to 4 groups of 6 rats each (Table 3.1). Group I which served as control was given normal saline orally for three weeks**,** group 2^{nd} served as diseased group was given single intradermal injection of FCA(0.1ml=100µg) at the base of tail to induce arthritis. Group 3^{rd} which served as treatment group 1 was given FCA(0.1ml=100µg) at the base of tail to induce arthritis + zingerone(25mg/kg b.w.; from the day of arthritis induction) in normal saline daily for 21 days using an oral gavage while group 4^{th} served as treatment group 2 was given single intradermal injection of FCA (0.1ml) at the base of tail +zingerone (25mg/kg b.w.; from day of arthritis onset) in normal saline daily for 21 days using an oral gavage.

Table 3.1: Experimental design of the study

Group 1 (Control Group)	N = 6	Normal saline orally
Group 2 (Diseased Group)	N = 6	Arthritic control rats (positive control) single intradermal injection of FCA(0.1ml) at the base of tail
Group 3 (Treatment Group I)	N= 6	Single intradermal injection of FCA 0.1ml at the base of tail + zingerone (25mg/kg b.w.; from the day of arthritis induction) in normal saline daily for 21 days using an oral gavage
Group 4 (Treatment group II)	N =6	Single intradermal injection of FCA 0.1ml at the base of tail + zingerone (25mg/kg b.w.; from day of arthritis onset) in normal saline daily for 21 days using an oral gavage

3.4 Measurement of clinical severity of Arthritis

The severity of arthritis in each affected paw was assessed by a macroscopic scoring system and was graded on a subjective scale of 1-3 as described by Banerjee *et al.,* (1988). The severity of arthritis in each affected paw was graded as: Grade 1, redness and swelling; Grade 2, deformity; and Grade 3, ankylosis in the affected joint. The maximum grades in the affected paws of each diseased mouse were added to give an arthritic score for the animal and manipulation of affected joints for ankylosis.

3.5. Sample collection

After completion of the experiment the animals were sacrificed under light ether anesthesia. Blood was drawn by cardiac puncture in clean and dry test tube,

left 30 minutes to clot and centrifuged at 3000 rpm (4^{o}C) for serum separation. The separated serum was stored at -20^{o}C until analyzed. The liver and knee joints were immediately removed and washed by ice cold saline solution and kept in normal saline at -20^{o}C until analyzed.

3.6 Liver homogenate and Post-mitochondrial supernatant (PMS) preparation

The liver from all animals was first aseptically removed and then homogenized in a chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (KCl; 1.17% w/v) using a homogenizer. This liver homogenate was used for the estimation of Lipid peroxidation. The homogenate was further centrifuged at 3000 rpm for 10 min at 4˚C to separate the nuclear debris. The supernatant was further centrifuged at 12,000 rpm for 20 min at 4˚C to get the post-mitochondrial supernatant (PMS), which was used for the estimation of antioxidant profile.

3.7 Preparation of Cell-Free Extract of the Knee Joints:

Arthritic and non-arthritic joints were removed and cut into small pieces and homogenized in 5 vol of 50mM TrisHCl buffer, pH 7.4 containing 0.1 M NaCl and 0.1% Triton X-100 and 1 vol. of fine glass powder by using a mortar and pestle. The crude extract then was sonicated for 20 sec. The homogenate was centrifuged at 5,500 rpm for 5min, and the resulting supernatant was stored at -80°C for estimation of LPO, immune markers and antioxidant profile.

3.8 Protein estimation:

Protein estimation of each sample of liver PMS and joint CFE was done by the method of Lowry *et al.,* (1951). Standard curve was obtained by using bovine serum albumin (50mg%).

3.9 Estimation of Lipid Peroxidation (LPO)

LPO was estimated by the method of Wright *et al.,* (1981). The reaction mixture, in a total volume of 2.0 ml, contained 1.8 ml, phosphate buffer (0.1 M, pH 7.4) and 0.2 ml of liver homogenate (10% w/v). The reaction mixture was incubated at 37˚C in a shaking water bath for 1 hr. The reaction was stopped by the addition of 1.0 ml trichloroacetic acid (TCA) (10% w/v). Following the addition of 1.0 ml thiobarbituric acid (TBA) (0.67% w/v, prepared in warm distilled water), all the tubes were placed in a boiling water bath for 20 min. The tubes were cooled on ice and centrifuged at 2,500 xg for 10 min. The supernatant containing the thiobarbituric acid reactive substances (TBA-RS) was assessed by measuring the optical density at 535 nm. The results were expressed as nmole of malondialdehyde (MDA) formed/g of tissue using a molar extinction coefficient of 1.56×10^5/ M /cm.

3.10 Measurement of SOD (Superoxide dismutase) activity

The SOD activity was measured by the method of Marklund and Marklund (1974). The reaction mixture consisted of 2·875 ml Tris–HCl buffer (50mM, pH 8.5), .025 ml Pyrogallol (24mM in 10mM HCl) and 100 µl PMS in a total volume of 3 ml. The enzyme activity was measured at 420nm and was expressed as units/mg protein. Here, one unit of enzyme is defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50 %.

3.11 Estimation of Glutathione Peroxidase (GPx) activity

Specific activity of enzyme was measured according to the procedure described by Mohandas *et al.,* (1984). The reaction mixture consisted of 1.44 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml of EDTA (1 mM), 0.1 ml of sodium azide (1 mM), 0.05 ml Glutathione Reductase (1IU/ml), 0.05 ml of glutathione (1 mM), 0.1 ml of NADPH (0.2 mM), 0.01 ml of hydrogen peroxide (0.25 mM) and 0.1 ml of PMS (10% w/v) in a final volume of 2 ml. The disappearance of

NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmole NADPH oxidized/min/mg protein by using the molar extinction coefficient of 6.22×10^3/M/cm.

3.12 Estimation of Catalase (CAT) Activity

Catalase activity was determined by the method of Claiborne (1985). PMS was used to assess the activity of catalase. The assay mixture consisted of 2.0ml phosphate buffer (0.05 M, pH 7.0), 0.95 ml hydrogen peroxide (H_2O_2) (0.019 M) and .05 ml PMS (10% w/v) in a total volume of 3.0 ml. The rate of change of absorbance per minute at 240 nm was recorded and catalase activity was expressed as μ mol H_2O_2 decomposed/min/mg protein using extinction coefficient of 0.081 x 10^3/ M/cm.

3.13 Estimation of Nuclear factor-kappaB (NF-κB)

NF-κB content translocated to nucleus was estimated by using an ELISA kit (NF-κB p65 ELISA, Invitrogen Corporation, CA, USA) in the serum and cell free extract of joint according to protocol provided by the manufacturer. Briefly, in a 96-well plate pre-coated with primary antibody, 50 micro liter samples was added and incubated for 90 min. After incubation, wells were aspirated and washed with washing buffer provided with the kit and then detection antibody was added in each well and incubated for 1 h at room temperature. After the washing again 100 micro liters of Avidin- HRP diluted with assay diluents was added per well and incubated for 30 min at room temperature. Wells were aspirated and washed 7 times with 250 microliter washing buffer/well. 100 microliter substrate/well was added and incubated for 15 min. at room temperature and then reaction was stopped by the addition of 50 micro liter 2 N H_2SO_4. Standard curve for the NF-kB p65 was also obtained with the same procedure (standard was provided with the kit). Plates were read at 450 nm in a Multiscan EX microplate reader (Thermo).

3.14 Estimation of Tumor necrosis factor alpha (TNF-α)

TNF-α was estimated by using an ELISA kit of eBioscience from the serum and cell free extract of joint according to protocol provided by the manufacturer. Briefly, in a 96-well plate pre-coated primary antibody, 100 µL samples was added and incubated overnight at 4^{o}C After incubation wells were aspirated and washed 3 times with buffer provided with the kit and then 100 µL detection antibody was added in each well. The tubes were sealed and incubated at room temperature for 1 hr. After incubation the wells were aspirated and washed 3 times with buffer and 100 µL Avidin-HRP was added, the plates were sealed and incubated at room temperature for 30 minutes. Wells were aspirated and washed 7 times with 250 micro liters washing buffer/well. 100 micro liter substrate/well was added and incubated for 15 min. at room temperature and then reaction was stopped by the addition of 50 micro liter stop solution. Standard curve for the TNF-α was also obtained with the same procedure (standard was provided with the kit). Plates were read at 450 nm in a Multiscan EX microplate reader (thermo).

3.15 Estimation of Transforming Growth Factor-beta (TGF- β)

TGF-β was estimated by using an ELISA kit of eBioscience from the serum and cell free extract of joint according to protocol provided by the manufacturer. Briefly, in a 96-well plate pre-coated primary antibody, 100 µl of samples acidified with 20 µl 1N HCl and neutralized with 20 µl NaOH was added and incubated overnight at 4^{o}C. After incubation wells were aspirated and washed 5 times with buffer provided with the kit and then 100 µL detection antibodies was added in each well. The tubes were sealed and incubated at room temperature for 1 hr. After incubation the wells were aspirated and washed 5 times with buffer and 100 µL of Avidin-HRP was added, the plates were sealed and incubated at room temperature for 30 minutes. Wells were aspirated and washed 7 times with 250 micro liters washing buffer/well. 100 microliter substrate/well was added and

incubated for 15 min. at room temperature and then reaction was stopped by the addition of 50 micro liter stop solution. Standard curve for the TGF- β was also obtained with the same procedure (standard was provided with the kit). Plates were read at 450 nm in a Multiscan EX micro plate reader (thermo).

3.16 Estimation of Interleukin-1 beta (IL-1β)

IL-1β was estimated by using an ELISA kit of Qayee-Bio (Korea), cat.No: QY-20844, from serum and cell free extract of joint according to protocol, provided by the manufacturer. Briefly, in a 96-well plate pre coated primary antibody, 10 µl of sample along with 40µl special diluent was added. Then 50µl of HRP-conjugate was added, the plates were sealed and incubated for 60 minutes at room temperature. The plates were aspirated and washed 5 times, after washing 50µl of chromogen solution A was added, followed by 50µl of chromogen solution B and incubated for 10 minutes at room temperature away from light. After 50µl/well of stop solution was added. Standard curve for IL-1β was obtained in with the same procedure (Standard was provided with kit). Plates were read at 450nm in a MultiScan EX microplate reader.

3.17 Estimation of Interleukin-6(IL-6)

IL-6 was estimated by using an ELISA kit of DiacloneSAS(France), cat.No. 860.0202.096, from the serum and cell free extract of joint according to protocol provided by the manufacturer. Briefly, in a 96-well coated plate pre-coated primary antibody, 100 µl standards and samples were added to the plate. Then, 50 µl of anti-murine IL-6 was added to all wells. Plate was sealed and incubated at room temperature for 3 hours. After incubation, wells were aspirated and washed 3 times with buffer provided with the kit and then 100µl avidin-HRP solution was added in each well. The wells were sealed and incubated at room temperature for 30 minutes.Wells were aspirated and washed 3 times using washing buffer. 100µl TMB substrate/well was added and incubated for 15 min. at room temperature and then reaction was stopped by the addition of 100µl of stop

solution. Standard curve for IL-6 was obtained in with the same procedure (Standard was provided with kit). Plates were read at 450nm in a MultiScan EX microplate reader.

3.18 Estimation of Interleukin-10(IL-10)

IL-10 was estimated by using an ELISA kit of Diaclone SAS (France), cat.No. 860.030.096, from the serum and cell free extract of joint according to protocol provided in the kit. Briefly, in a 96-well coated plate pre-coated primary antibody, 100 µl standards and samples were added to the plate. Then, 50 µl of anti-murine IL-10 was added to all wells. Plate was sealed and incubated at room temperature for 3 hours. After incubation, wells were aspirated and washed 3 times with buffer provided with the kit and then 100µl Streptavidin-HRP solution was added in each well. The wells were sealed and incubated at room temperature for 30 minutes. Wells were aspirated and washed 3 times with washing buffer. 100µl TMB substrate/well was added and incubated for 15 min. at room temperature and then reaction was stopped by the addition of 100µl of stop solution. Standard curve for IL-10 was obtained in with the same procedure (Standard was provided with kit). Plates were read at 450nm in a MultiScan EX microplate reader(thermo).

3.19 Estimation of high sensitivity C - reactive protein (Hs-CRP):

Hs-CRP was estimated from the serum and cell free extract of joint by using an ELISA kit of Diaclone SAS (France), according to protocol provided by the manufacturer. Briefly, in a 96-well coated plate pre-coated primary antibody, 100 µl standards and samples were added to the plate. The Plate was sealed and incubated at 37° C for 90 minutes and after incubation, the plate contents were discarded and wells were washed 2 times with wash buffer. Then, 100 µl of Biotin- labeled antibody working solution was added to all wells. Plate was sealed and incubated at 37° C for 1 hour. After, incubation wells were aspirated and washed 3 times with buffer provide with the kit and then 100µl of HRP-

streptavidin conjugate working solution was added in each well. The wells were sealed and incubated at 37° C for 30 minutes .Wells were aspirated and washed 5 times using washing buffer. 90µl TMB substrate/well was added and incubated for 15 min. at room temperature and then reaction was stopped by the addition of 50µl of stop solution. Standard curve for Hs-CRP was obtained in with the same procedure (Standard was provided with kit). Plates were read at 450nm in a MultiScan EX microplate reader.

3.20. Statistical analysis:

The data from individual groups was presented as means with their standard errors. Differences between groups were analyzed by using ANOVA followed by the Tukey-Kramer multiple comparison test, by using primer software.

Chapter-4

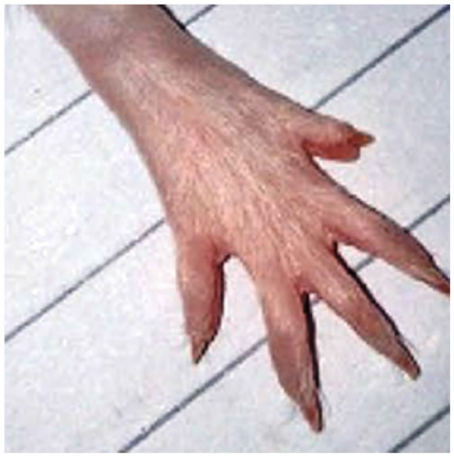
EXPERIMENTAL FINDINGS

4.1 Effect of Zingerone on Clinical Severity of Arthritis

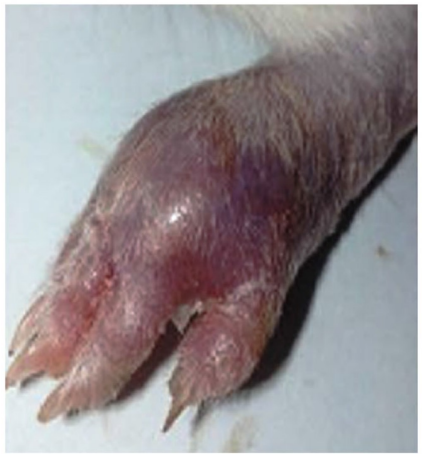
After injecting Complete Freund's adjuvant (CFA) 0.1ml (=100µg) intra-dermally for induction of arthritis, early clinical arthritic signs of inflammation were observed in rats on day 10 after immunization with CFA. Redness, hotness, or swelling was observed in the joints. All the signs peaked by day 17. Arthritic rats (Diseased control) showed an increase in thickness in paws and ankles which were evidenced by increased inflammation, edema and ankylosis. The extent of inflammation and edema of paw was mild in zingerone treated group III (treatment 1) rats as compared to the diseased control (group II) while zingerone treated group IV (treatment II) also exhibited a decrease in inflammation and there were no signs of ankylosis when compared to disease control (group II) (Fig.4.1).

4.2 Effect CFA and Zingerone treatment on Lipid peroxidation in liver and joint tissues

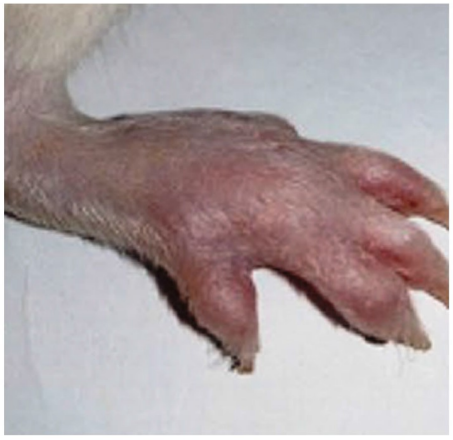
Malondialdehyde (MDA) is the stable product of lipid peroxidation there by indicating the membrane damage.The administration of CFA resulted in marked increase ($P < 0.01$) and ($P < 0.001$) of MDA content in liver and joint tissue respectively with respect to their controls (table 4.1, Fig. 4.2). The treatment with zingerone significantly reduced the elevated hepatic levels of MDA content in group III compared with group II (disease control), the group IV treated with zingerone also showed reduction in the hepatic MDA content in comparison to diseased control but the reduction was statistically non-significant, however the treatment with zingerone resulted in significant reduction in MDA content of joint in both group III and IV compared to disease control, the significance was more pronounced in group III than group IV.



Group I
(Normal Control)



Group II
(Disease Control)



Group III
(Treatment I)

Group IV
(Treatment II)

Fig 4.1: Effect of zingerone on clinical severity of arthritis

4.2 Effect ofCFA and Zingerone treatment on various antioxidants in liver and joint tissues

The concentration of SOD, glutathione peroxidase and catalasewas evaluated in hepatic and joint tissue to estimate the endogenous defence mechanism against superoxide anions generated by lipid peroxidation due to administration of CFA. The CFA administration resulted in significant (<.01) reduction in SOD activity in both hepatic and joint tissue in comparison to control (group I), the administration of zingerone significantly (<.01 and <.05) increased the activity of SOD in hepatic and joint tissue in group III compared to group II (disease control) (table4.2, Fig. 4.3).The administration of zingerone in group IV improved the level of SOD in both hepatic and joint tissue as compared to group II but the elevation was statistically non-significant.

Table 4.1: Effect of CFA and Zingerone on LPO in liver and joint tissue

Expt.Grps.	**LPO Liver (nmol MDA formed/g tissue)**	**LPO knee joint (nmol MDA formed/g tissue)**
Group I	2.28±0.18	1.72±0.14
Group II	$3.31\pm0.23^{**}$	$2.72\pm0.21^{***}$
Group III	$2.56\pm0.12^{\#}$	$1.93\pm0.09^{\#\#}$
Group IV	2.82 ± 0.16^{ns}	$2.22\pm0.17^{\#}$

Values are expressed as mean ± 6SE; n=6 animals in each group. *indicates significance from control group. $^{\#}$indicates significance from the CFA group. ***indicates significance at P<.001. $^{\#\#}$indicates significance at P<.01. $^{\#}$indicates significance at P<.05. ns indicates non-significant.

Table 4.2: Effect of CFA and Zingerone on antioxidant enzymes in liver and joint tissue

Expt.Grps.	SOD Liver (Units/mg protein)	GPx Liver (nmol NADPH oxidized/min/mg protein)	CAT Liver (nmol H_2O_2 consumed /min/mg protein)	SOD Joint (Units/mg protein)	GPxJoint (nmol NADPH oxidized/min/mg protein)	CAT Joint (nmol H_2O_2 consumed/min/mg protein)
Group I	4.48±0.49	101±15.2	10.98±0.17	2.15±0.29	44.8±0.58	20.81±0.45
Group II	$2.11\pm0.30^{**}$	$48\pm4.8^{***}$	$5.90\pm0.25^{***}$	$0.85\pm0.09^{**}$	$18.6\pm0.22^{***}$	$5.45\pm0.29^{***}$
Group III	$4.40\pm0.70^{\#\#}$	$89\pm3.4^{\#\#}$	$10.53\pm0.11^{\#\#}$	$1.17\pm0.15^{\#}$	$31.7\pm0.37^{\#\#\#}$	$17.63\pm0.37^{\#\#}$
Group IV	2.55 ± 0.33^{ns}	62 ± 5.8^{ns}	$10.24\pm0.34^{\#}$	1.01 ± 0.04^{ns}	$25.5\pm0.22^{\#\#\#}$	$16.78\pm0.42^{\#\#}$

Values are expressed as mean ± 6SE; n=6 animals in each group. *indicates significance from control group. $^{\#}$indicates significance from the CFA group. ***indicates significance at P<.001. $^{\#\#}$indicates significance at P<.01. $^{\#}$indicates significance at P<.05. ns indicates non-significant.

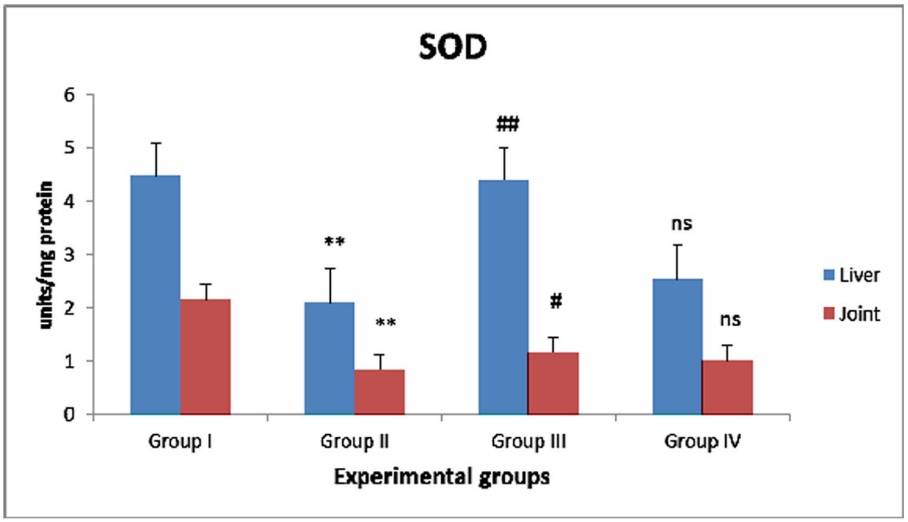


Fig 4.3: Effect of CFA and Zingerone on SOD in liver and joint tissue

Values are expressed as mean ± 6SE; n=6 animals in each group. ⋆ indicates significance from control group. #indicates significance from the CFA group. ⋆⋆indicates significance at P<.01. ##indicates significance at P<.01. #indicates significance at P<.05. ns indicates non-significant.

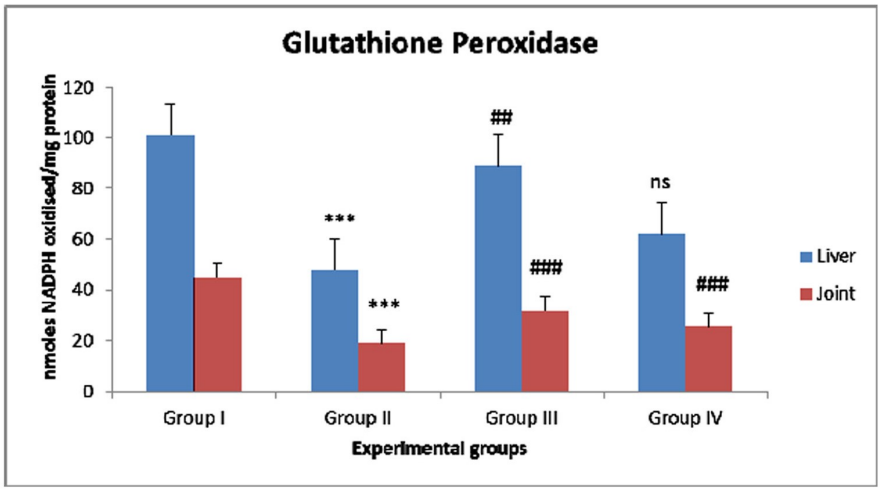


Fig 4.4: Effect of CFA and Zingerone on GPx in liver and joint tissue

Values are expressed as mean ± 6SE; n=6 animals in each group. ⋆ indicates significance from control group. #indicates significance from the CFA group.*⋆⋆indicates significance at P<.001. ###indicates significance at P<.001. ##indicates significance at P<.01. ns indicates non-significant.

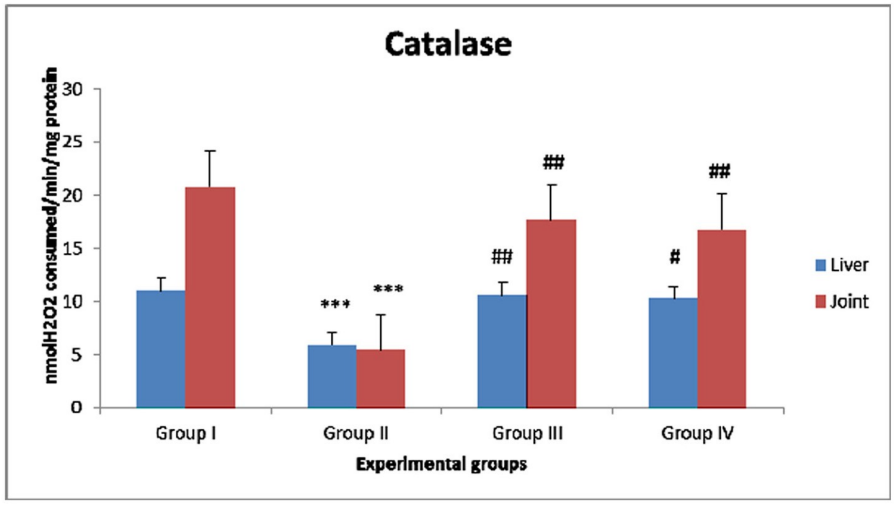


Fig 4.5: Effect of CFA and Zingerone on Catalase in liver and joint tissue

Values are expressed as mean ± 6SE; n=6 animals in each group. ⋆ indicates significance from control group. #indicates significance from the CFA group.*⋆⋆indicates significance at P<.001. ###indicates significance at P<.001. ##indicates significance at P<.01. ns indicates non-significant.

Glutathione peroxidase (GPx) activities were also assessed to check H_2O_2 mediated damage resulted due to administration of FCA. The administration of CFA resulted in significant (<.001) reduction in the activity of GPx in both hepatic and joint tissues (group II) respectively compared to normal control (group I). Zingerone administration significantly (<.01) enhanced the hepatic GPx activity in group III as compared to group II, however there was no significant difference in the hepatic GPx activity in group IV (treatment II) after zingerone supplementation as compared to group II. The administration of zingerone significantly (<.001) elevated the activity of GPx in joints of both group III and IV tissue compared to group II (Fig. 4.4).

The catalase activity was significantly (<.001) reduced in both hepatic and joint CFA treated group (group II) as compared to group I (normal control). Zingerone treatment significantly augmented the activity of catalase in both hepatic and joint of group III and group IV as compared to group II (table 4.2, Fig. 4.5).

4.3 Effect of CFA and Zingerone treatment on joint and serum NF-κB levels

Nuclear factor NF-κB is a pleiotropic transcription factor and works as one of the key supervisors for triggering and amplifying inflammation in arthritis. In the present study, the administration of CFA induced significant (<.001) increase in the level of NF-kB in joint and serum of group II as compared to group I (control). Treatment with zingerone significantly (<.001) decreased the levels of NF-κB in the joints of both group III and IV as compared to group II (Table 4.3, Fig.4.6).

The levels of NF-κB in the serum was significantly (<.01) reduced in group III experimental animals as compared to group II animals, however the treatment with zingerone in group IV animals did not exhibited any significant difference from group II animals (Table 4.4, Fig. 4.7).

Table 4.3: **Effect of CFA and Zingerone on immune markers in joint CFE**

Experimental groups	**NF-κB (pg/g protein)**	**TNF-α (pg/g protein)**	**TGF-β (pg/g protein)**	**IL-1β (pg/g protein)**	**IL-6 (pg/g protein)**	**Hs-CRP (pg/g protein)**	**IL-10 (pg/g protein)**
Group I	68.40±0.36	31.17±1.30	20.067±0.74	41.83±5.34	105.8±10.2	12.69±1.43	10.47±0.28
Group II	$95.42\pm0.34^{***}$	$67.50\pm3.66^{***}$	$29.78\pm2.16^{**}$	$74.67\pm7.25^{**}$	$248.3\pm31.0^{**}$	$22.16\pm1.30^{**}$	$8.6\pm0.54^{**}$
Group III	$74.37\pm0.15^{\#\#\#}$	$35.33\pm1.15^{\#\#\#}$	$21.93\pm0.59^{\#\#}$	$47.33\pm5.30^{\#\#}$	$156.7\pm21.4^{\#\#}$	$15.64\pm1.23^{\#\#}$	$18.62\pm0.25^{\#\#\#}$
Group IV	$82.85\pm0.31^{\#\#}$	$38.08\pm2.70^{\#\#\#}$	$23.38\pm1.05^{\#\#}$	65.00 ± 6.39^{ns}	82.5 ± 23.5^{ns}	16.69 ± 1.99^{ns}	$11.83\pm0.44^{\#\#}$

Values are expressed as mean ± 6SE; n=6 animals in each group. *indicates significance from control group. $^{\#}$indicates significance from the CFA group. ***indicates significance at P<.001. $^{\#\#}$indicates significance at P<.01. $^{\#}$indicates significance at P<.05. ns indicates non-significant.

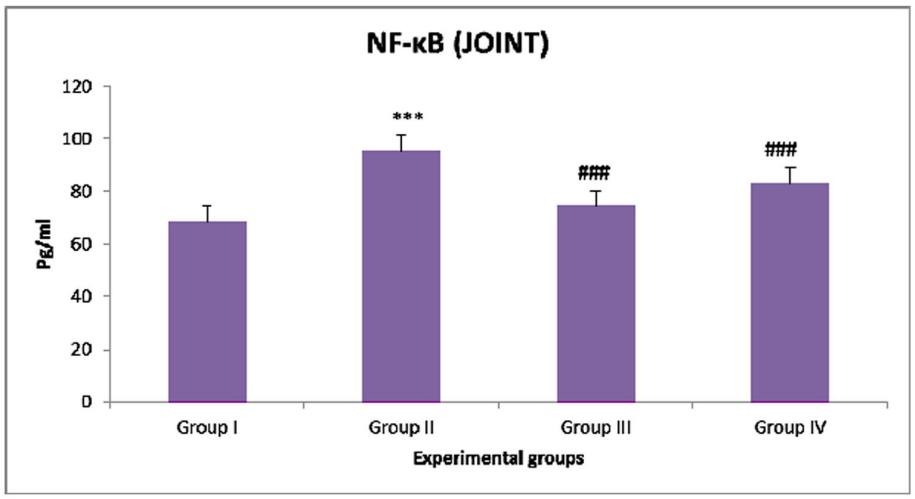


Fig.4.6: Effect of CFA and Zingerone on NF-κB in Joint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. ***indicates significance at P < .001. ###indicates significance at P < .001. ns indicates non-significant.

Table 4.4: **Effect of CFA and Zingerone on immune markers in serum**

Experimental groups	NF-κB (pg/ml protein)	TNF-α (pg/ml protein)	TGF-β (pg/ml protein)	IL-1β (pg/ml protein)	IL-6 (pg/ml protein)	Hs-CRP (pg/ml protein)	IL-10 (pg/ml protein)
Group I	62.13±0.11	31.73±1.72	17.83±1.35	35.83±5.19	104.2±20.3	3.75±0.31	16.07±0.19
Group II	$84.07\pm0.47^{***}$	$60.67\pm3.68^{***}$	$25.62\pm2.17^{*}$	$67.33\pm8.09^{**}$	$221.7\pm28.6^{***}$	$7.22\pm0.40^{***}$	$14.54\pm0.49^{**}$
Group III	$73.14\pm0.27^{\#\#}$	$34.33\pm1.50^{\#\#\#}$	$18.23\pm1.32^{\#}$	$41.70\pm5.07^{\#\#}$	$132.50\pm6.16^{\#\#\#}$	$4.38\pm0.23^{\#\#\#}$	$26.65\pm0.14^{\#\#\#}$
Group IV	82.75 ± 0.46^{ns}	$45.33\pm2.26^{\#\#\#}$	21.73 ± 2.31^{ns}	56.67 ± 6.62^{ns}	171.67±9.28	$4.78\pm0.28^{\#\#}$	$20.4\pm0.42^{\#\#}$

Values are expressed as mean ± 6SE; n=6 animals in each group. *indicates significance from control group. $^{\#}$indicates significance from the CFA group. ***indicates significance at P<.001. $^{\#\#}$indicates significance at P<.01. $^{\#}$indicates significance at P<.05. ns indicates non-significant.

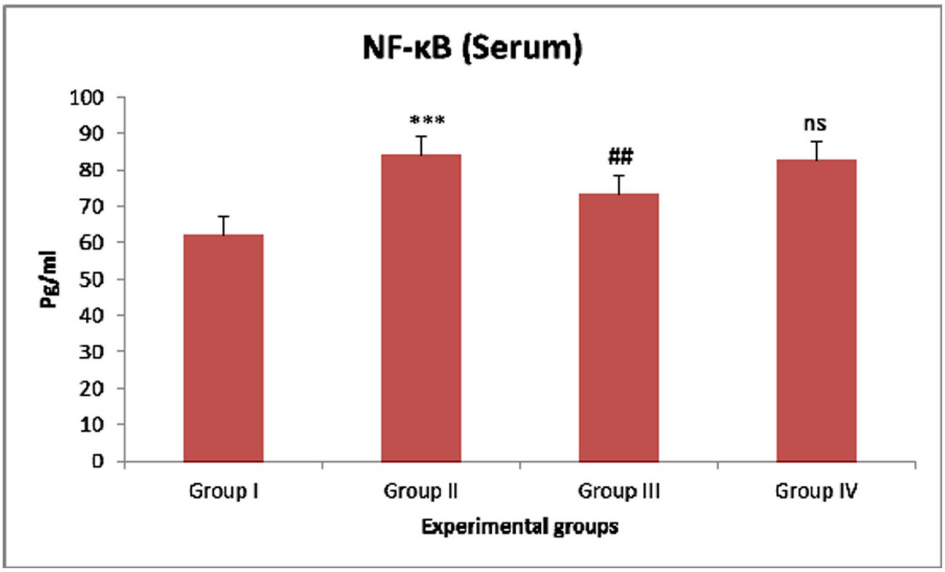


Fig.4.7: Effect of CFA and Zingerone on NF-κB in Serum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. ***indicates significance at P < .001. ##indicates significance at P < .01. ns indicates non-significant.

Nuclear factor NF-κB is a pleiotropic transcription factor and works as one of the key supervisors for triggering and amplifying inflammation in arthritis. In the present study, the administration of CFA induced significant (<.001) increase in the level of NF-kB in joint and serum of group II as compared to group I (control). Treatment with zingerone significantly (<.001) decreased the levels of NF-κB in the joints of both group III and IV as compared to group II (Table 4.3, Fig.4.6).

4.4 Effect of CFA and Zingerone treatment on joint and serum TNF-α levels

Levels of inflammatory cytokine, TNF-α were measured as a marker of inflammation. The intoxication with CFA resulted in significant (<.001) increase in the joint and serum levels of TNF-α in group II as compared to group I (control). The supplementation of zingerone significantly (<.001) reduced the levels of pro-inflammatory TNF-α levels in joint and serum of group III and IV as compared to group II (FCA (Table4.3, 4.4 and Fig. 4.8 & 4.9) .

4.5 Effect of FCA and Zingerone treatment on joint and serum TGF-β levels

The administration of CFA resulted in significant (<.01) increase in the levels of TGF-β in the joints of group II experimental animals as compared to group I animals (control). The treatment with zingerone significantly ameliorated the levels of TGF-β in the joints of group III and group IV animals as compared to group II (disease control) (Table 4.3,Fig. 4.10).

Further, in the present study, the administration of CFA resulted in significant (<.01) increase in the concentration of TGF-β levels in serum of group II animals as compared to the group I(control) animals. The supplementation of zingerone significantly (<.05) reduced the serum levels of TGF-β in treatment group III, however the treatment with zingerone did not result in significant alteration in TGF-β levels in group IV animals as compared to group II animals (Table 4.4, Fig. 4.11).

4.6 Effect of CFA and Zingerone treatment on joint and serum IL-1β levels

Cytokine IL-1β is reported to play a critical role in the pathogenesis of RA.In the present study, immunization with CFA resulted in significant (<.01) increase in

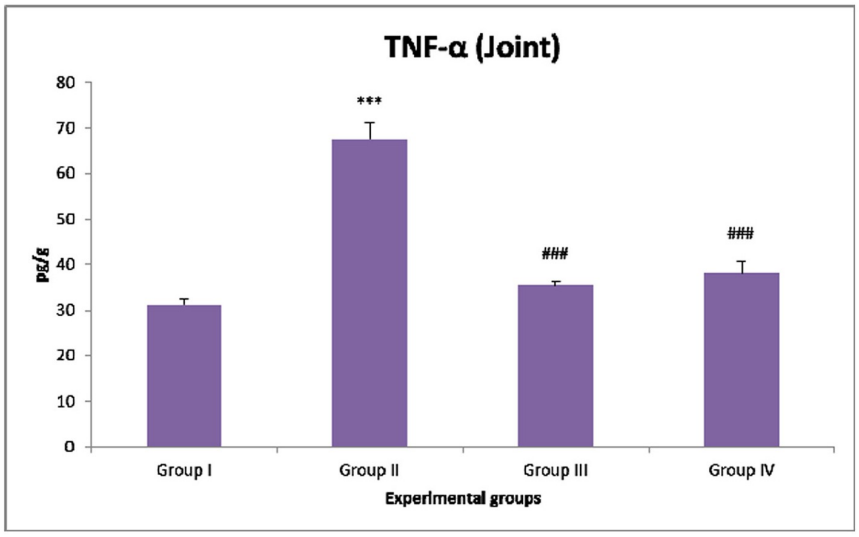


Fig.4.8: Effect of FCA and Zingerone on TNF-α in Joint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. ***indicates significance at P < .001. ###indicates significance at P < .001. ns indicates non-significant.

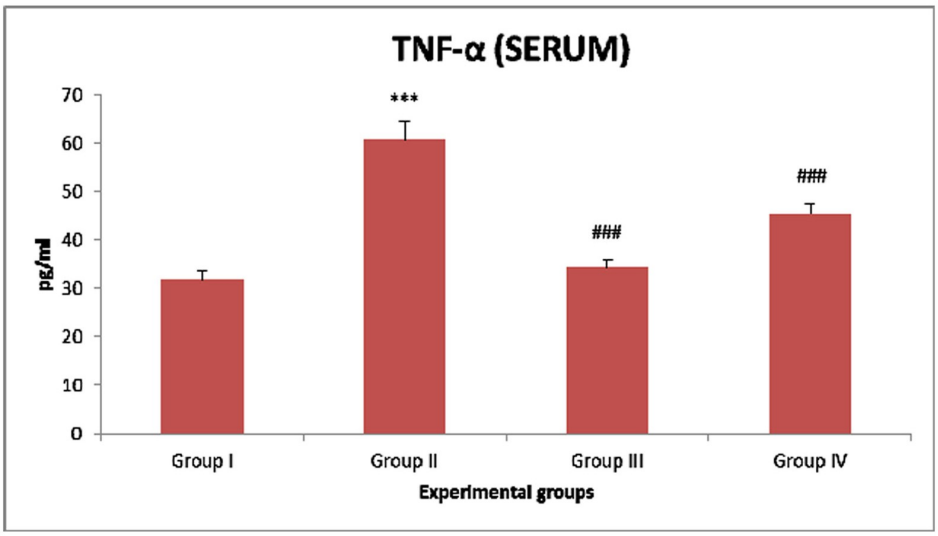


Fig.4.9: Effect of CFA and Zingerone on TNF-α in Serum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. ***indicates significance at P < .001. ###indicates significance at P < .001. ns indicates non-significant.

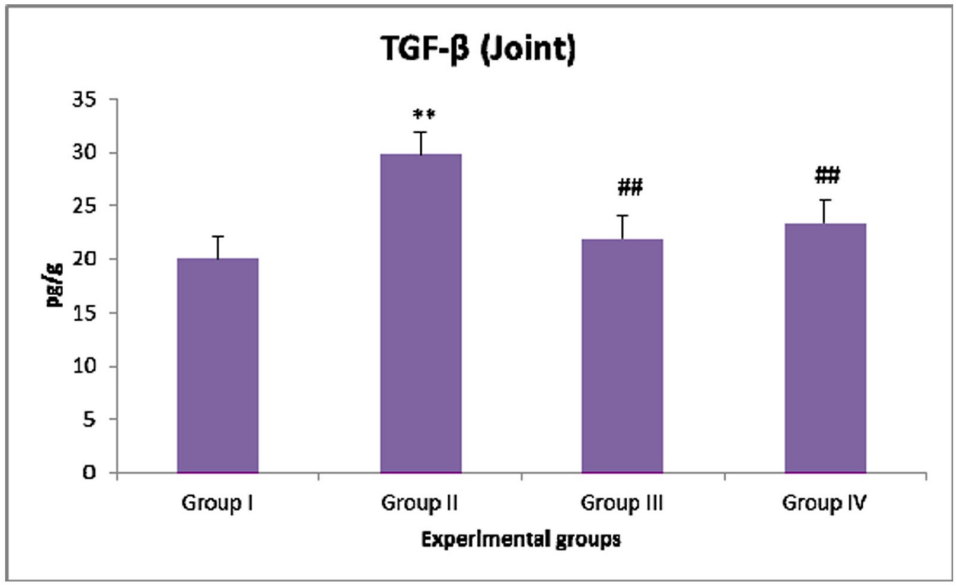


Fig.4.10: Effect of CFA and Zingerone on TGF-β in Joint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group.# indicates significance from the CFA group. **indicates significance at P < .01. ##indicates significance at P < .01. ns indicates non-significant.

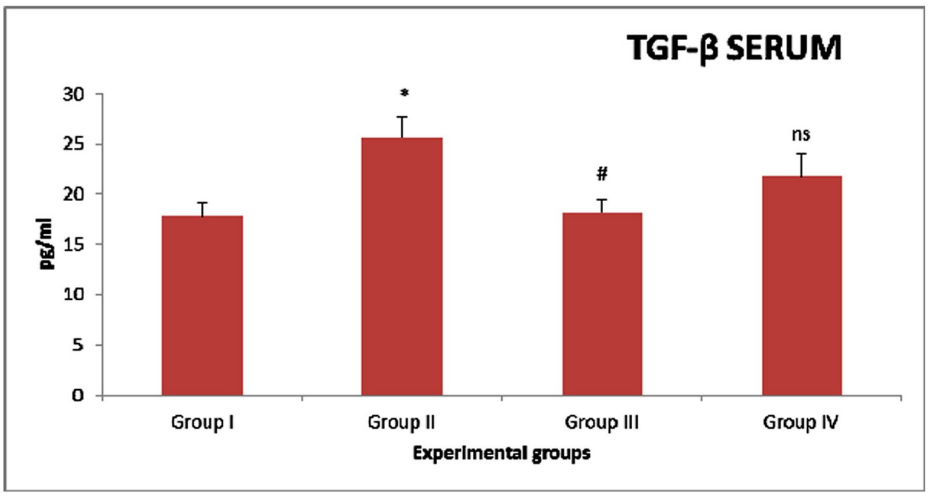


Fig.4.11: Effect of CFA and Zingerone on TGF-β in Serum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. *indicates significance at P < .05. #indicates significance at P < .05. ns indicates non-significant.

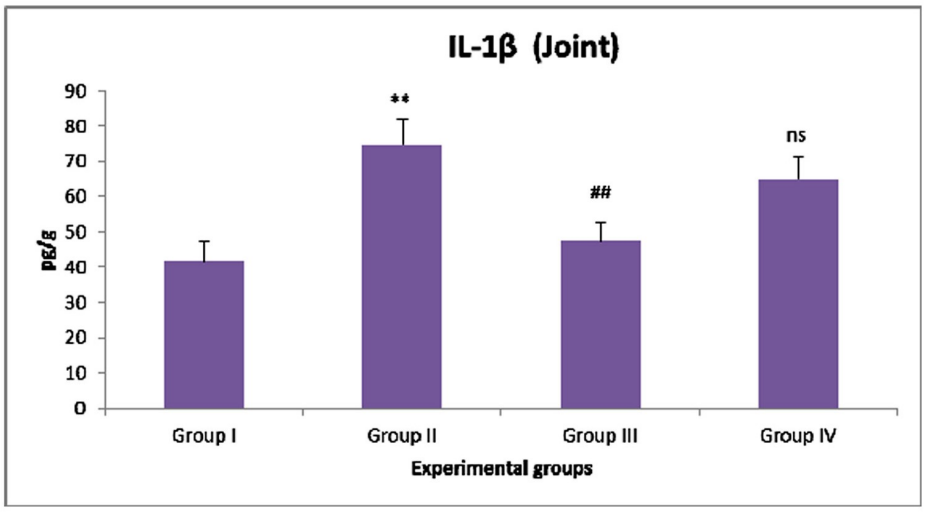


Fig. 4.12: Effect of CFA and Zingerone on IL-1β in Joint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. **indicates significance at P < .01. ##indicates significance at P < .01. ns indicates non-significant.

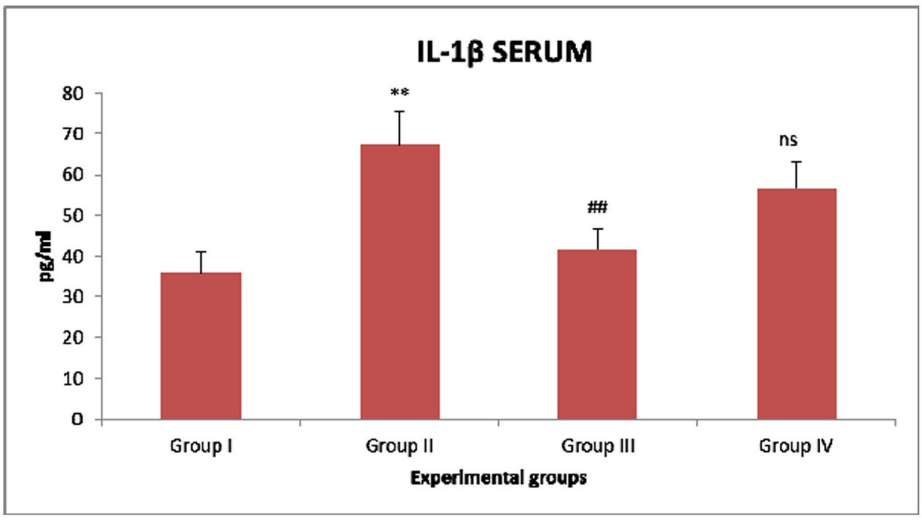


Fig.4.13: Effect of CFA and zingerone onIL-1β inSerum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. **indicates significance at P < .01. ##indicates significance at P < .01. ns indicates non-significant.

the joint and serum levels of IL-1β in group II animals as compared to the group I animals (control). The zingerone supplement significantly (<.01) restored the IL-1β levels in joints and serum of group III and group IV animals as compared to group II animals (Table 4.3, 4.4 and Fig. 4.12 & 4.13).

4.7 Effect of CFA and Zingerone treatment on joint and serum IL-6 levels

The inflammatory cytokine IL-6 was measured as a marker of inflammation. The treatment with CFA resulted in significant (<.01) increase in the concentration of IL-6 levels in the joints of group II animals as compared to group I. The supplementation of zingerone significantly (<.01) restored the levels of IL-6 in the joints of treatment group III as compared to group II, however the zingerone treatment did not significantly altered the levels of IL-6 in joints of group IV as compared to group II (Table 4.3, Fig.4.14).

The administration of CFA resulted in significant (<..001) increase in the serum levels of IL-6 in group II as compared to group I (control). The zingerone supplementation significantly (<.001) ameliorated the levels of serum IL-6 in group III treated animals as compared to group II animals, however there was no significant alteration in the serum levels of IL-6 in group IV as compared to group II after zingerone supplementation (Table 4.4, Fig. 4.15)

4.8: Effect of CFA and Zingerone treatment on joint and serum Hs-CRP levels

High sensitivity C - reactive protein, an exquisitely sensitive, acute phase systemic marker of inflammation, is used as an index for the measurement of inflammation in the body. Joint C-RP levels of CFA injected group II rats were significantly (<.001) increased as compared with group I(control). The supplementation of zingerone significantly (<.01) reduced the levels of Hs-CRP in group III rats as compared to group II but in group IV the zingerone

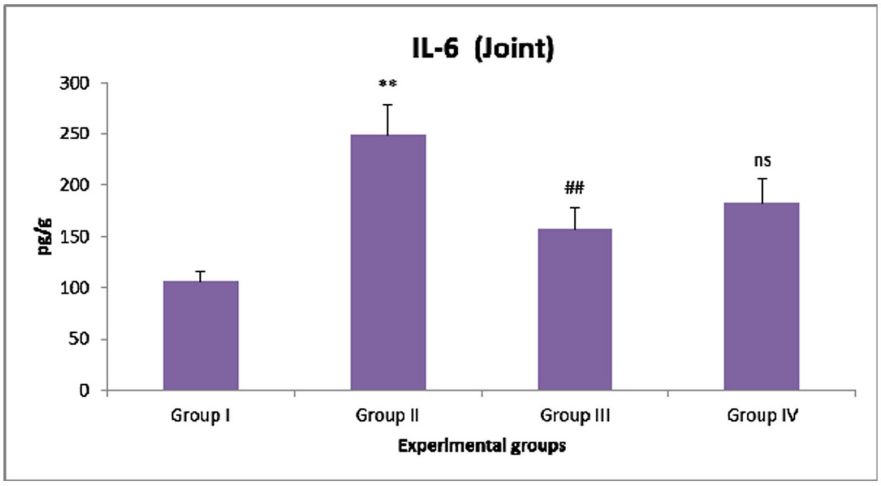


Fig.4.14: Effect of CFA and Zingerone on IL-6 in Joint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. **indicates significance at P < .01. ##indicates significance at P < .01. ns indicates non-significant.

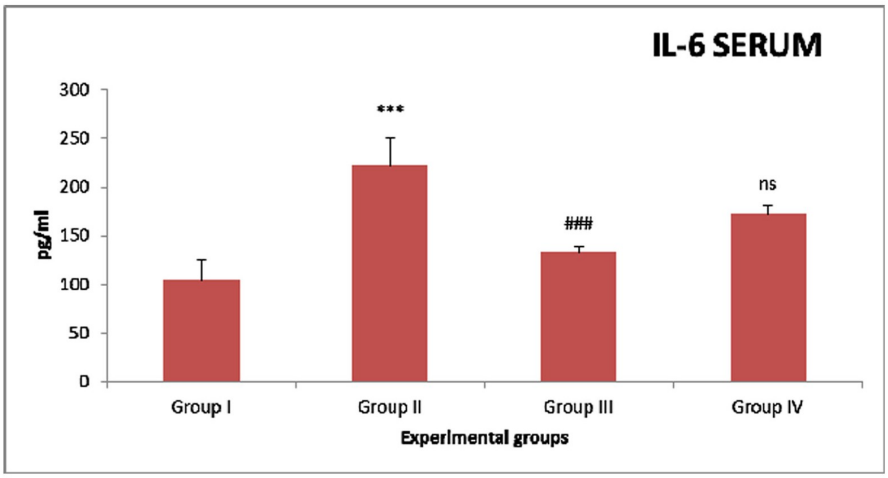


Fig.4.15: Effect of CFA and Zingerone on IL-6 in Serum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. ***indicates significance at P < .001. ###indicates significance at P < .001. ns indicates non-significant.

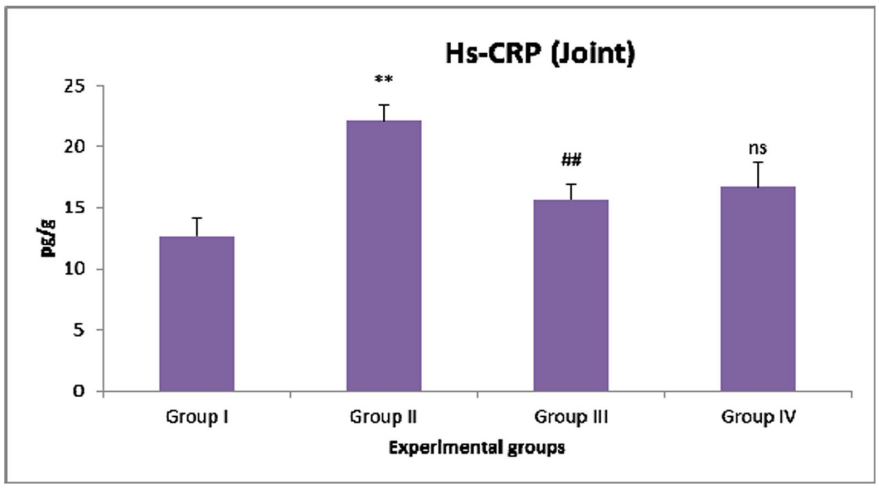


Fig.4.16: Effect of CFA and Zingerone on Hs-CRP in Joint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. **indicates significance at P < .01. ##indicates significance at P < .01. ns indicates non-significant.

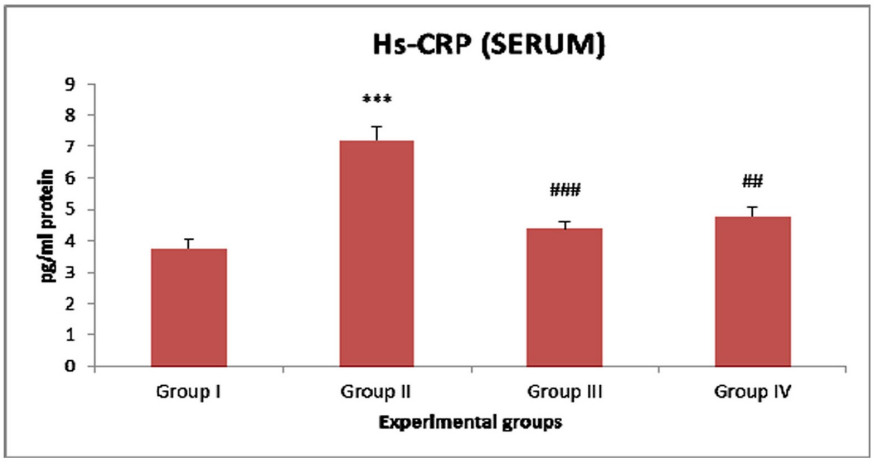


Fig.4.17: Effect of CFA and Zingerone on Hs-CRP in Serum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group.# indicates significance from the CFA group. ***indicates significance at P < .001. ###indicates significance at P < .001. ##indicates significance at P < .01. ns indicates non-significant.

supplementation did not altered the levels of Hs-CRP significantly as compared to group II (Table 4.3, Fig. 4.16).

The administration of CFA resulted in significant (<.001) increase in the Hs-CRP levels of serum in group II animals as compared to group I (control). The supplementation with zingerone significantly decreased the Hs-CRP levels in both the treated groups (group III and group IV) as compared to group II however the significance was more pronounced in group III (Table 4.4, Fig. 4.17).

4.9: Effect of CFA and Zingerone treatment on joint and serum IL-10 Levels

The concentration of IL-10 was evaluated to estimate endogenous defenses against inflammation. In the present study the CFA administration resulted in the significant (<.01) reduction of IL-10 levels in both joint and serum levels of group II as compared to group I. The zingerone administration significantly increased the IL-10 levels in joint and serum of group III and group IV as compared to group II, the significance was more in group III (Table 4.3, 4.4 and Fig. 4.18 & 4.19).

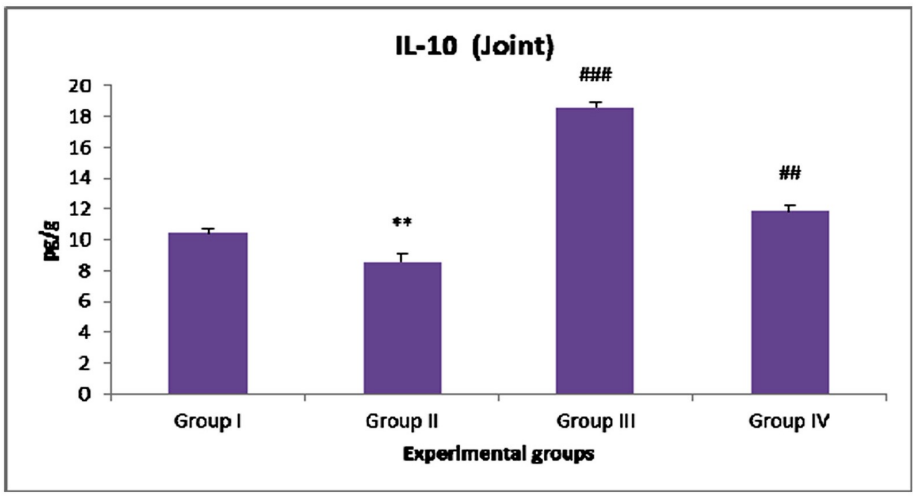


Fig.4.18: Effect of CFA and Zingerone on IL-10 inJoint Cell Free Extract

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. **indicates significance at P < .01. ###indicates significance at P < .001. ##indicates significance at P < .01. ns indicates non-significant.

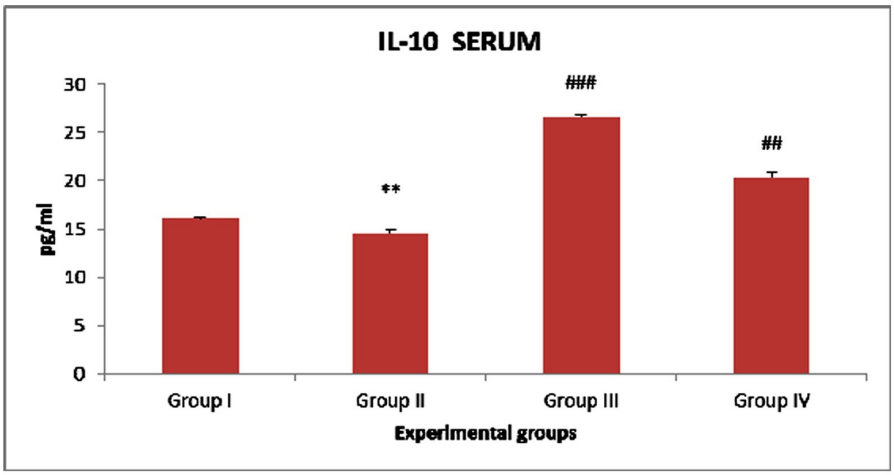


Fig.4.19: Effect of CFA and Zingerone on IL-10 in Serum

Values are expressed as mean 6± SE; n = 6 animals in each group. * indicates significance from control group. # indicates significance from the CFA group. **indicates significance at P < .01. ##indicates significance at P < .01.###indicates significance at P < .001. ns indicates non-significant.

Chapter-5

DISCUSSION

Arthritis is a group of diseases considered to encompass more than a hundred inflammatory or degenerative conditions. Arthritis is not only a disease of joints, but also associated with immune, hepatic, renal and other organ systems damage that directly or indirectly affects the joints. Hence, it is required to determine the pathological and biochemical aspects of arthritis that are necessary in evaluating the activity of drugs (Rainsford, 1982). Osteoarthritis and RA are two common types of arthritis characterized by joint inflammation, immune cell infiltration, synovial hyperplasia, joint pain and swelling that result in the destruction of joint integrity and function disability (Neugebauer *et al*., 2007). RA is a chronic inflammatory disease affecting about 1% of the population in developed countries (Amresh *et al*., 2007). Because of the similarities in clinical features, the adjuvant-induced arthritis in rat is extensively used for evaluating the efficacy of anti-inflammatory drugs in rheumatoid RA (Noguchi *et al*., 2005).

CFA-induced arthritis is well-recognized model and has been commonly used for the evaluation of anti-inflammatory and anti-arthritic potential of various agents(Costa *et al*., 2004).CFA is composed of mineral oil inactivated and dried mycobacteria, that contain different pathogen-associated molecular patterns including toll-like receptor 2, 4, and 9 agonists and responsible for stimulation of cell-mediated immunity that increases the synthesis of certain immunoglobulin's (Singh *et al*., 1996). The intra-dermal administration of CFA into the tail of rats leads to reactivity to heat shock proteins, cartilage proteoglycans, and interactions with intestinal flora (van Vollenhoven *et al*., 1988). Further, release of diverse inflammatory mediators such as, cytokines, lysosomal enzymes, hydrolytic enzymes and prostaglandins (PGs) known to participate in the pathogenesis of RA (Naik and Wala, 2014).

The traditional use of plants as medicines provide the basis for indicating which essential oils may be useful for specific medical conditions. Also the resurgence

of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitativedata on plant oils and extracts are required. Because current treatments for arthritis result in unwanted side effects and tend to be expensive, natural products devoid of such disadvantages offer a novel opportunity. Agents derived from plants that can modulate the expressionof pro-inflammatory signals clearly have potential against arthritis.

In the present study, with the aim to develop a safe and an effective anti-arthritic agent, Zingerone was evaluated for its Preventive and Therapeutic role in Adjuvant-induced Arthritis in experimental rats, by intervening with the inflammatory pathways and oxidative processes.

Reduction in paw swelling is an index of measurement of anti-arthritic activity of various drugs (Rajendran and Krishnakumar, 2010). The measurement of paw swelling is a simple, quick and sensitive method for evaluating intensity of inflammation (Kweifio-Okai and Carroll, 1993). RA presents with edema of periarticular tissues such as ligaments and joint capsules. The intensity of ligament swelling and joint capsule swelling increases in the initial phase of inflammation. These changes in the paw volume are associated with an increase in the accumulation of granulocytes and monocytes in the joint tissue (Arend and Dayer ,1990). In the chronic inflammatory phase, the activation of macrophages results in the production of several cytokines such as IL-6 and TNF-α that have been associated with immune arthritis (Yu *et al*., 2006). In the present study, zingerone measured parameters revealed anti-arthritic effect. The significant increase in the paw thickness after intra-dermal administration of CFA is reflective of the phase of arthritis. The oral treatment of zingerone showed decrease in the paw thickness, arthritic score and joint diameter by inhibiting the release of inflammatory mediators, indicating its anti-inflammatory potential in CFA induced arthritis model. The treatment of rats with CFA exhibited elevated thickness of the hind paw. After CFA immunization, the arthritis swelling in the right hind paws of rats was increased and maintained for 21 days compared with

NC group. However, the treatment of rats with zingerone inhibited the paw swelling as compared to CFA group.

Nuclear factor NF-κB is a pleiotropic transcription factor and works as one of the key supervisors for triggering and amplifying inflammation in RA (Morel and Berenbaum, 2004; Tu *et al*., 2005). In the basal state, NF-κB exists in the cytoplasm complex with the inhibitory-kB (IκB) proteins. On stimulation, phosphorylation of IκB by inhibitory-κB kinase (IKK) triggers its degradation and the activation of NF-κB. On activation, NF-κB translocates to the nucleus and starts the synthesis of various inflammatory mediators such as TNF-α and iNOS. Therefore, modulation of NF-κB activity either through inhibition of NF-κB translocation to the nucleus or through inhibition of DNA binding activity of NF-κB, could be an important strategy to reduce inflammation and cellular injury. Several studies reported that zingerone reduces NF-κB activation with a consequent reduction in cell damage (Kim *et al.,* 2010). Thus, this study examined the attenuating effect of zingerone on NF-κB activity in AIA rats. In line with the above discussion, in the present study, increased levels of NF-κB activity were found in AIA rats, which decreased after treatment with zingerone. This supports the hypothesis that the probable mechanism of action of zingerone in reducing arthritic joint inflammation is intimately related with either the in-vivo inhibition of DNA binding activity of NF- κB or inhibition of NF-κB translocation to the nucleus. As NF-κB regulate innate and adaptive immune system through transcription of inflammatory markers such as TNF-α, IL-1β, IL-6, and metabolic enzyme nitric oxide synthase and COX. Activation of NF-κB worsens the RA (Lawrence,2009). After CFA immunization, TNF-α, IL-6, and IL-1β are released from macrophages and monocytes. Tumor necrosis factor alpha (TNF-α) is a chief inflammatory mediator that can stimulate the secretion of numerous other inflammatory cytokines such as IL-1β and IL-6, which result in the transport of more leukocytes, infiltration, and vasodilatation at the site of edema. In addition, TNF-α mediated activation of the NF-κB, establishes a positive feedback loop of this destructive cycle (Filippin *et al.,* 2008). Furthermore, these pro-inflammatory

cytokines stimulate chemokines, which attract neutrophils and monocytes towards inflamed joints (Voon *et al*. 2017). To avoid bone and cartilage destruction, it is necessary to block pro-inflammatory cytokines (TNF-α) involved in gene expression of matrix metalloproteinase (MMPs) (Srirangan and Choy, 2010). This study expressed a marked decrease in the expression of TNF-α, IL-1β, and IL-6 in contrast to the arthritic control group. So our findings are in concurrence with the findings of Rehman *et al*., 2018 that treatment with zingerone markedly abrogated ROS levels, inhibited the NF-κB activation and considerably reduced level of other downstream inflammatory molecules (TNF-α, IL-6, IL-1β)in a streptozotocin/high fat diet (STZ/ HFD)-induced type 2 diabetic Wistar rat model

IL-10 is the immune-regulatory cytokine, which halts the process of inflammation, bone erosion, and cartilage damage during RA in contrast to inflammatory cytokines by suppressing the activation of IKK and so NF-κB (Schottelius *et al*., 1999). IL-10 not only diminishes pro-inflammatory T helper cell generated cytokines mainly TNF-α and IL-1, but also maintains the integrity of joint tissues during the RA (Shabbir *et al*., 2014). The results of the present study showed a raised level of IL-10 in treatment groups, in contrast to the arthritic control group.

TGF-β is a pleiotropic immune-regulatory cytokine found up-regulated in RA (Pohlers *et al*., 2007). Even though TGF-β is considered an anti-inflammatory molecule, in some pathologic conditions, it may have a reversed activity as a pro-inflammatory cytokine (Cheon *et al*., 2002; Ahmad *et al*., 2017). In RA in particular, elevated TGF-β levels in plasma and synovial fluid have been reported (Fava *et al*., 1989). In the present study, significant elevation of TGF-β in the arthritic untreated group relative to the normal control group and the decreased level with zingerone treatment indicates that it may play arole as a pro-inflammatory cytokine in RA. So our findings are in concurrence with the above findings.

Moreover, serum CRP is the biomarker of systemic inflammation, which represents active inflammation. The CRP level is positively correlated with

severity and progression of rheumatoid arthritis (Rhodes *et al*., 2011). Increased level of CRP indicates the progression of arthritis. Elevated level of IL-6 and TNF-α aggravates the production of CRP as evidenced from the previous studies (Kumar *et al*., 2013). In the present study, an increased CRP level was observed in CFA-treated rats and treatment with zingerone reversed the CFA-induced CRP changes and reduced systemic inflammation as depicted by a low level of CRP via reduction of IL-6 and TNF-α, as shown in the previous studies (Kalaiselvan and Rasool, 2016).

Oxidative stress contributes to the pathogenesis of RA. Excessive generation of ROS produced by activated neutrophils and macrophages inflicts damage to joints mainly through up regulation of matrix metallo-proteinases and activation of osteoclast activity (Mirshafiey and Mohsenzadegan,2008). Above normal concentrations, reactive oxygen species (ROS) damage lipids, proteins, membranes, nucleic acids; activate nuclear factor kappa-β (NF-κB); induce pro-inflammatory cytokines (Sukkar and Rossi, 2004); and trigger connective tissue destruction within the joint(Kurien and Scofield, 2008).

Peroxidation of lipids could dramatically change the propertiesof biological membranes, resulting in severe cell damage andplays a significant role in the pathogenesis of disease. Additionally, it has been shown that certain lipid peroxidationproducts induce genetic over-expression of proteins such as cytokines (Halliwell *et al*., 1988). Zingerone is reported to inhibit LPO and to possess SOD like activity (Krishnakantha and Lokesh ,1993). Similarly, in the present study, higher levels of liver and joint MDA or lipid peroxides were observed in CFA-injected rats which was significantly decreased on treatment with zingerone, which is in agreement with previous reports (Ahmad *et al*., 2018; Rajkumar and Rao,1994; Oboh *et al*.,2012;).

Hydrogen peroxide generated as a result of SOD activity is decomposed by ubiquitous protein Catalase (CAT) and Glutathione Peroxidase (GPx) into water and molecular oxygen. Under oxidative stress, the activity of GPx and CAT increases due to an increase of the maximum enzyme reaction rate (Km) or may

be due to oxidant-induced transcriptional upregulation of GPx and CAT expression (Pandey and Rizvi, 2010; Dieterich *et al*., 2000). As observed in the present study and some previously reported studies, oxygen free radicals are responsible for the increase in GPx and CAT activity in AIA rats, as compared to controls. These results suggest an adaptive response in AIA rats to the increase in oxidative stress, and could be a consequence of a process of enzyme induction. These imbalances in the activity of enzymatic antioxidants are ameliorated by treatment with zingerone. This result suggests that zingerone has an antioxidant activity in AIA and is responsible for the decrease in the production of ROS, which finally may inhibit the tissue damage caused due to oxidative stress. These findings are in agreement with the previous reports of Hemalatha and Prince, (2015); Rajan *et al*., (2013); Rao and Rao, (2010); Rahmani *et al.*, (2014).

The significant decrease/increase in the cellular toxicity markers (LPO, and anti-oxidant defence system (SOD, CAT and GPx), respectively, shown in arthritic rats that received zingerone especially from the day of arthritis induction, emphasizes the role of zingerone in preventing organs damage and bone loss in AIA rat model through scavenging the free radicals.

Chapter-6

SUMMARY AND CONCLUSION

Complete Freund's adjuvant-induced arthritis is one of the best animal models of chronic polyarthritis with features resemble to human rheumatoid arthritis and involved cell mediated autoimmunity. The Freund's adjuvant model is chosen, as it develops chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction. Because current treatments for arthritis result in unwanted side effects and tend to be expensive, natural products devoid of such disadvantages offer a novel opportunity. Indeed, several preclinical and clinical studies suggest that the large number of inexpensive natural products that can modulate inflammatory responses, but lack side effects, constitute 'goldmines' for the treatment of arthritis. Zingerone is a pharmacologically active ingredient that is present in dried ginger and is known to have therapeutic effects such as antioxidant, anti-inflammatory, anti-obesity, oxidative stress antagonist, anti-nausea and anti-vomiting agent after chemotherapy, and antidiuretic agent. In order to explore the natural compound zingerone as potent anti-inflammatory and anti-oxidant agent against arthritis, thecurrent study was designed to evaluate the Preventive and Therapeutic Role of Zingerone in Adjuvant-induced Arthritis in wistar rats.

Rats were randomly divided into 4 groups, 6 rats in each group. Group I was normal control, group II was arthritic group, Group III was arthritic treated with zingerone at the dose rate of 25 mg/kg b.wt., from the day of arthritis induction, Group IV was arthritic treated with zingerone at the dose rate of 25 mg/kg b.wt., from the day of arthritis onset. Freund's Complete adjuvant 0.1ml (=100µg) was given by intra-dermal injection for induction of arthritis. Except group I all the three groups were given FCA for induction of arthritis. The zingerone was given from day 0 and from day 11, after the induction of arthritis for a period of 21 days through oral route in group III and group IV respectively. Animals at the end of

study were sacrificed using Diethyl ether anesthesia and blood samples were collected by cardiac puncture in clot activator vials. Liver and knee joints were obtained for Post Mitochondrial Supernatant and Cell Free Extract preparation, respectively. The results obtained in the present study are summarized as,

- ✓ Immunization with CFA presented severe physical arthritic symptoms. Zingerone notably mitigated these physical arthritic changes by reducing the extent of inflammation and edema of paw and ankle in group III and group IV rats.
- ✓ Zingerone showed anti-inflammatory properties in CFA induced arthritis by down regulating the levels of pro-inflammatory cytokines, NF-κB, TNF-α, TGF-β, IL-6, IL-1β and increasing anti-inflammatory cytokine IL-10 in group III and group IV.
- ✓ Moreover, Zingerone reduced systemic inflammation as depicted by a low level of C - reactive protein in group III and group IV.
- ✓ CFA inoculation caused oxidative stress by decreasing the activity of various anti-oxidant enzymes like SOD, CAT, GPx & increasing lipid peroxidation. Zingerone significantly increased the activities of SOD, GPx and CAT and decreased lipid peroxidation of membranes by decreasing the levels of MDA and revealed potent antioxidant properties in arthritic rats.
- ✓ However, Zingerone was more effective in alleviating the inflammatory immune response and oxidant stress, in group III i.e., when the treatment started from the day of arthritis induction.
- ✓ Therefore, Preventive (Prophylactic) effect of Zingerone was more profound when compared to Therapeutic (Curative) value.
- ✓ Further studies are warranted to study the exact mechanism involved in ameliorative effects of zingerone in arthritis.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Sandeep Kumar Sr. Scientist Veterinary Biochemistry LUVAS Hisar Haryana** during viva voce examination held on **05-02-2020** have been incorporated in the manuscript entitled **,"To investigate the Preventive and Therapeutic Role of Zingerone in Adjuvant-induced Arthritis in Experimental Rats"** submitted by **Dr. Nazirah Bashir (Regd. No. 2017-V-354-M).**

Dr. Sheikh Bilal Ahmad

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