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Zingerone (4-(four-hydroxy-3-methylphenyl) butane-two-1) modulates adjuvant-induced rheumatoid arthritis by regulating inflammatory cytokines and antioxidants

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ABSTRACT

Objective: Ginger (*Zingiber officinale* Roscoe) is considered to be one of the most commonly consumed dietary condiments of the world. The present study was designed to explicate the protective role of zingerone; an active ingredient of ginger in complete Freund's adjuvant (FCA)-immunized arthritic rats.

Methods: 24 Wistar rats were divided into 4 groups with 6 rats each. Group I as control followed by group II, III and IV were treated with single intradermal injection of FCA (0.1 ml = 100 µg) to induce rheumatoid arthritis. Group III and IV were also administered with zingerone orally at 25 mg/kg b.w for 3 weeks at two different time points.

Results: Adjuvant-treated rats exhibited a significant increase in lipid peroxidation and a reduction in the enzymatic antioxidants such as SOD, catalase and GPx, in the liver and joint tissues. Moreover, FCA inoculation resulted in the increase in levels of NF-κB, TGF-β, TNF-α, IL-1β, IL-6 and Hs-CRP and a decrease in IL-10 levels. Zingerone significantly reduced the levels of NF-κB, TGF-β, TNF-α, IL-1β, IL-6 and Hs-CRP and markedly increased IL-10 levels. Levels of antioxidant enzymes were also restored by zingerone treatment.

Discussion: Oral administration of zingerone ameliorated inflammatory outburst and decreased oxidative stress, suggesting its role in the prevention of rheumatoid arthritis. Further mechanistic insights are necessary to study the exact mechanism involved.

KEYWORDS
FCA; zingerone; inflammatory markers; Hs-CRP; oxidative stress; rheumatic arthritis; phytomedicines; cytokines

Introduction

Arthritis is a disease with multiple factors and has been reported to affect nearly 1% adult population of the world [1, 2]. It is a progressive inflammatory disease resulting in the destruction of bone and cartilage through the involvement of synovial membranes of joints [3, 4]. Although the main target of disease process in arthritis is joints, the disease is recognized as a non-organ-specific autoimmune disease, because of the occurrence of extra-articular signs, such as subcutaneous nodules, vasculitis and pulmonary fibrosis [5]. The initial phase of the disease results in strong inflammation of joints by the recognition of self-protein epitopes by auto-reactive T and B cell clones [6]. The activated Th1 and Th17 cells coordinate this process by the secretion of cytokines. The cytokines, in turn, activate innate immune cells leading to pain, bone degradation and reduction in cartilage repair [7, 8].

Many cytokines contribute to rheumatoid arthritis and act as the key factors responsible for the loss of ability of tissues forming joints by activating destructive processes [9]. Tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and interleukin one beta (IL-1β) are the key inflammatory molecules responsible for the pathophysiological alterations taking place during the course of rheumatoid arthritis and increased levels of these inflammatory markers are observed in synovial fluid, synovial membrane, cartilage and sub-chondral bone layer [10]. Moreover, in joint cells, IL-1β induces its own secretion and stimulates synthesis of other molecules such as TNF-α, interleukin six (IL-6), interleukin eight (IL-8), and CCL chemokine [11, 12].

During the disease process, IL-1β stimulates reactive oxygen species (ROS) production along with decreased expression of oxidative enzymes, thereby accelerating further damage of articular cartilage in the joint affected by the disease [13]. TNF-α and IL-1β act in a synergic manner in many phenomena that take place in the course of rheumatoid arthritis [14]. This effect is the result of the activation of similar intracellular signaling pathways, which trigger inflammation and destruction of joint tissues [15]. So these reactive oxygen species (ROS) and mediators of inflammation act in complete coordination.

A number of drugs that are being currently used to relieve pain and inflammation of damaged joint include non-

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steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying anti-rheumatoid drugs (DMARDs), biologicals such as TNF-α and IL antagonists [16–18]. However, these drugs have lost their role because of the safety concerns and associated side effects such as gastrointestinal or cardiac toxicity [19, 20], immune suppression, osteoporosis, and metabolic disorders [21, 22], high incidence of infection rate and huge cost [23, 24].

Due to the limitations of the above therapeutic approaches, there is a need for the identification of biomolecules from plants or natural sources devoid of toxic effects. The medicinal plants and herbs are playing an essential role in the health and vitality of human beings and animals. Hence, several phytomedicines (medicinal plants or herbs) are now used for the prevention and cure of arthritis. In the recent past, many of these herbs and compounds obtained from plant sources have received attention as an alternate source of medicine [25].

Zingiber officinale is a monocotyledonous medicinal plant, native to India or Southeast Asia, from where it reached to rest of the world [26]. Both fresh and dried ginger has found its use as a food additive and dietary spice as well as a phytomedicines [27]. Several studies have reported the effectiveness of compounds isolated from ginger against inflammatory diseases [28]. Zingerone, one of the active components, isolated from *Zingiber officinale*, is a phenolic alkanone that contains a vanilloid (4-hydroxy-3-methoxyphenyl) group in its structure with many pharmacological properties including antioxidant [29], anti-inflammatory [30], anticancer [31], antimicrobial [32] and antidiabetic activity [33].

Since no literature is available on the potential role of zingerone on rheumatoid arthritis, the current study was contemplated as a first time investigation to decipher the mechanisms involving the promising anti-arthritic effect of zingerone by studying inflammatory markers, oxidative stress, and arthritic markers in an experimental model of Freund's Complete Adjuvant (FCA)-induced rheumatoid arthritis.

Materials and methods

Experimental animals

Six- to eight-week-old, albino rats (140–160 g) of Wistar strain used in the study were purchased from Indian Institute of Integrated Medicine (IIIM) Jammu. The protocols were approved by the 'Institutional Animal Ethical Committee (IAEC)' (Vide no: AU/FVSc/VCC/1-3/19/815-16 dated: 23-11-2019) accredited by CPCSEA, New Delhi, India. Animals were kept in polypropylene cages in groups of three rats per cage at 25°C and had free access to standard diet and water.

Preparation of zingerone

Oral dose was prepared by dissolving 0.5 g of zingerone (Sigma Aldrich) in 100 ml of normal saline [34].

Design of experiment

After acclimatization period the animals were divided into 4 groups with 6 rats in each group to assess the effect of zingerone on FCA-induced rheumatoid arthritis. Group I served as normal control and received diet + water *ad libitum* and normal saline orally for three weeks**,** group II which served as disease control, received single intra-dermal injection of FCA (0.1 ml = 100 µg) at the base of tail to induce rheumatoid arthritis. Group III (treatment group I) received FCA (0.1 ml) at the base of tail + zingerone (25 mg/kg b.w.; from the day of arthritis induction) in normal saline daily for three weeks by an oral gavage, while group IV (treatment group II) received single intra-dermal injection of FCA (0.1 ml) at the base of tail + zingerone (25 mg/kg b.w.; from day of arthritis onset) in normal saline daily for three weeks by an oral gavage (Table 1).

Table 1. Tabular design of experiment.

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

Measurement of arthritis

The degree of severity of arthritis was assessed in the affected paw by macroscopic scoring and grading system, as described by Banerjee et al. [35]. In each affected paw, the severity of arthritis was graded on a subjective scale of 1–3 as redness and swelling (grade 1), deformity (grade 2) and ankylosis (grade 3). The progress of arthritis was assessed by measuring the paw thickness with the help of calipers.

Sample collection

After completion of the experiment all animals were sacrificed with light ether anesthesia. Blood was drawn by cardiac puncture and centrifuged at 3000 rpm (4°C) for the collection of serum. Liver and knee joints were removed for the preparation of homogenate and cell-free extract.

Liver homogenate and preparation of post-mitochondrial supernatant (PMS)

After removing the liver aseptically from all animals, it was homogenized in a chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (KCl; 1.17% w/v). The homogenate was used for lipid peroxidation (LPO) analysis. The homogenate was further centrifuged at 800× *g* for 5 min at 4°C in order to separate the nuclear debris. The supernatant was further centrifuged at 10000× *g* for 20 min at 4°C to get the post-mitochondrial supernatant for the analysis of antioxidant profile.

Preparation of cell-free extract of the knee joints

Knee joints from arthritic and non-arthritic rats were removed and cut into small pieces with the help of a blade and homogenized in 5 vol of 50 mM Tris HCl buffer, pH 7.4 containing

0.1 M NaCl and 0.1% Triton X-100 and 1 vol. of fine glass powder with the help of a mortar and pestle. The crude extract was sonicated for 20 sec. The homogenate was then centrifuged at 3000× *g* for 5 min, and the resulting supernatant was used for the estimation of LPO, immune markers and antioxidant profile.

Estimation of malondialdehyde (MDA)

LPO in liver homogenate and cell-free extract of joint was estimated by Wright et al. [36]. LPO was expressed as nmoles of MDA formed/g tissue.

Estimation of superoxide dismutase (SOD) activity

The activity of SOD in post-mitochondrial supernatant of the liver and cell-free extract of joint was assayed by the method of Marklund and Marklund [37] and was measured as units/mg protein.

Estimation of glutathione peroxidase (GPx) activity

The activity of GPx in post-mitochondrial supernatant of the liver and cell-free extract of joint was measured by Mohandas et al. The enzyme activity was recorded as nmoles of NADPH oxidized/mg protein [38].

Estimation of catalase activity

Catalase activity in PMS of the liver and cell-free extract of joint was done by the method of Claiborne and the enzyme activity was measured as nmoles of H_2O_2 consumed/min/mg protein. [39].

Estimation of nuclear factor-kappaB (NF-κB)

NF-κB was assessed by rat NF-κB ELISA-based kit (NF-κB p65 ELISA, Invitrogen Corporation, CA, USA) in the serum as pg/ml and cell-free extract of joint in pg/g tissue. The assay was carried out as per the instructions of the manufacturer.

Estimation of TNF-α and TGF-β

TNF-α and TGF-β levels were estimated using rat TNF-α and TGF-β ELISA-based kits of (eBioscience San Diego CA, USA) from the serum and cell-free extract of joint, respectively. The TNF-α and TGF-β levels were estimated as per the instructions of the manufacturer and were measured as pg/mg in cell-free extract of joint and as pg/ml in serum.

Estimation of interleukin-1 beta (IL-1β)

IL-1β levels were assayed by rat IL-1β ELISA-based kit (Qayee-Bio Korea). The estimation of IL-1β levels was carried out as per the instructions of the manufacturer and was measured as pg/mg in cell-free extract of joint and as pg/ml in serum.

Estimation of IL-6, IL-10 and high-sensitivity C-reactive protein (Hs-CRP)

IL-6, IL-10 and Hs-CRP were estimated using rat IL-6, IL-10 and Hs-CRP ELISA-based kits (DiacloneSAS, France) from the serum and cell-free extract of joint as per the directions of the manufacturer. The estimation of IL-6, IL-10 and Hs-CRP was carried out as per the directions of the manufacturer and was measured as pg/mg in cell-free extract of joint and as pg/ml in serum.

Statistical analysis

The experimental data obtained were expressed as mean ± standard deviation (SD). Differences between groups were analyzed by using one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls Test and the minimum criterion for difference was set at $P < 0.05$.

Results

Effect of zingerone and FCA on clinical severity of arthritis

After injecting FCA for the induction of rheumatoid arthritis, early symptoms of inflammation were visible in rats on day 10 after immunization with FCA. The affected joints showed redness, hotness, or swelling. All these signs peaked by day 17. Arthritic rats (disease control) showed an increase in thickness in paws and ankles which were evidenced by severe inflammation, edema and ankylosis. The inflammation and edema of paw was mild in zingerone-administered group III (treatment I) rats compared to diseased group II (diseased group), while zingerone-administered group IV (treatment II) also exhibited a decrease in inflammation and there were no signs of ankylosis compared to disease control.

Effect of zingerone and FCA on hepatic and joint oxidative stress parameters

Immunization with FCA resulted in a significant ($P < 0.01$ and $P < 0.001$) increase in the MDA levels of hepatic and joint tissue, respectively compared to the control group (Figure 1(A)). Zingerone treatment resulted in the reduction in the elevated hepatic MDA levels in group III and elevated MDA levels in joints of both the treatment groups compared with group II (disease control). FCA immunization significantly reduced ($P < 0.01$) the levels of SOD in hepatic and joint tissues of group II (disease control) compared to group I (normal control) (Figure 1(B)). However, zingerone treatment significantly elevated the levels of SOD in hepatic and joint tissue of group III compared to disease control, while the group IV rats did not show any significant elevation of SOD in hepatic and joint tissue in comparison to group II rats. Furthermore, adjuvant treatment significantly reduced ($P < 0.001$) the activity of GPx and catalase in disease control in comparison to group I (Control). Zingerone treatment augmented the activities of GPx and catalase in both the treatment groups (Figure 1(C,D)).

Effect of zingerone and FCA on inflammatory cytokine, TGF-β and Hs-CRP in joint

NF-κB, TNF-α, IL-1β, IL-6, TGF-β and Hs-CRP are the key players of inflammation and play a critical role in rheumatoid arthritis. Immunization with FCA significantly ($P < 0.001$, $P <$ 0.001and $P < 0.01$) increased the NF-κB, TNF-α and TGF-β

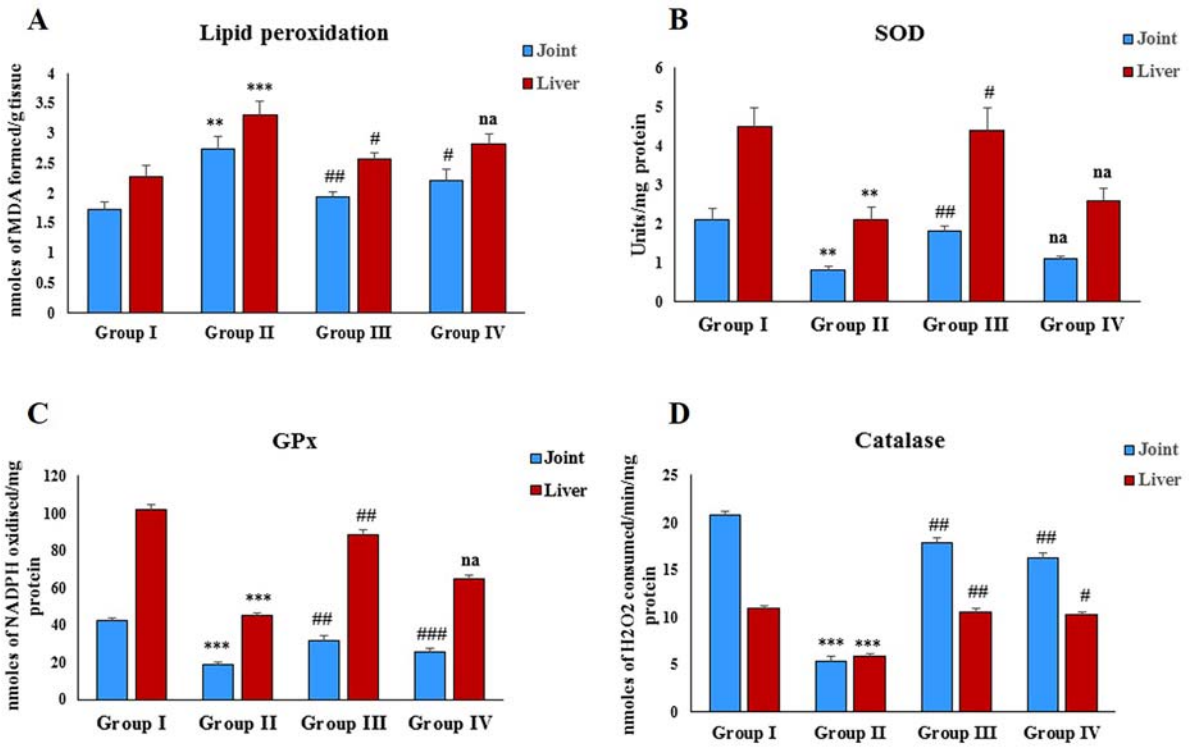

Figure 1. Effect of CFA and zingerone on LPO and activity of SOD, GPx and catalase. Values are presented as mean ± SD; $n = 6$ animals in each group. **indicates significance at $P < 0.01$ from the control group. ***indicates significance at $P < 0.001$ from the control group. #indicates significance at $P < 0.05$ from the CFA group. ##indicates significance at $P < 0.01$ from the CFA group. ###indicates significance at $P < 0.001$ from the CFA group. na = non-significant. LPO was expressed as nmoles of MDA formed/g tissue, SOD as units/mg protein, Gpx as nmoles of NADPH oxidized/mg protein and catalase as nmoles of H_2O_2 consumed/min/mg protein.

levels in the disease control group compared to the normal control group (Figure 2(A–C)). Administration of zingerone significantly attenuated these levels in both the treatment groups compared to the disease control (group II). Treatment with FCA also resulted in a significant ($P < 0.01$) increase in the levels of IL-1β, IL-6 and Hs-CRP in the disease control (group II) compared to the control (Figure 3(A,B,D)) and a significant ($P < 0.01$) decrease in the levels of IL-10 (Figure 3(C)). Administration of zingerone reduced the levels of IL-1β, IL-6, Hs-CRP in the treatment group 1 compared to the disease control;

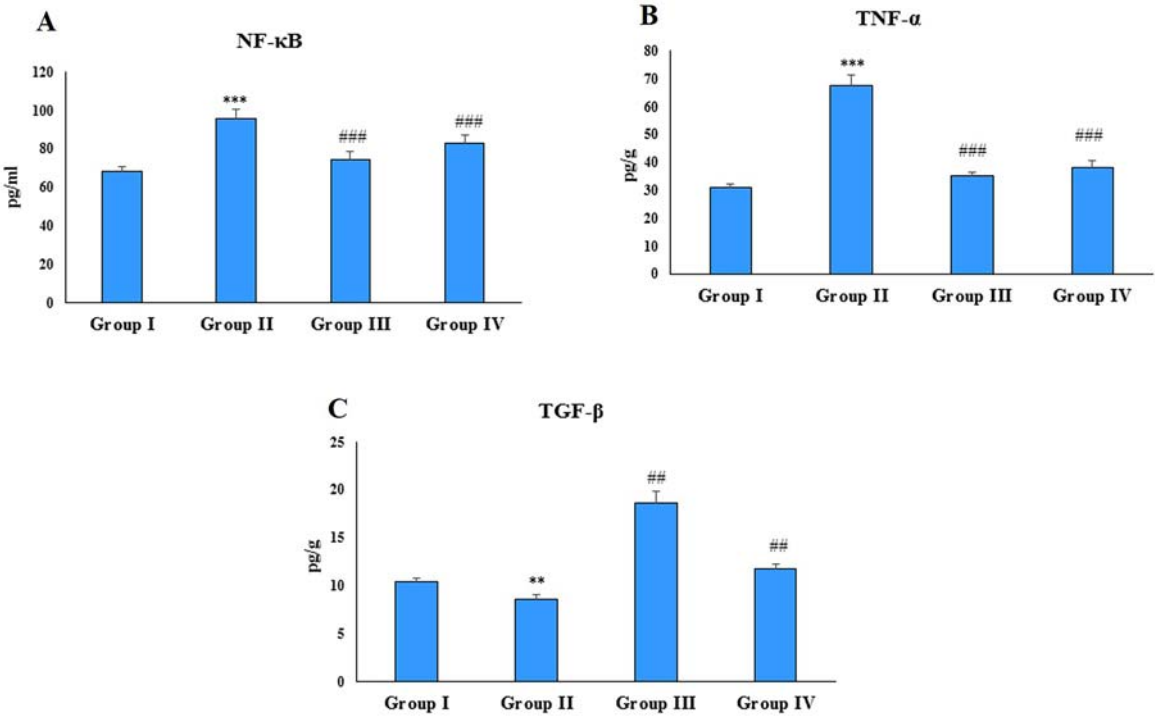

Figure 2. Effect of CFA and zingerone on NF-κB, TNF-α and TGF-β in cell-free extract of joint. Values are presented as mean ± SE; $n = 6$ animals in each group. **indicates significance at $P < 0.01$ from the control group. ***indicates significance at $P < 0.001$ from the control group. ##indicates significance at $P < 0.01$ from the CFA group. ### indicates significance at $P < 0.001$ from the CFA group. NF-κB, TNF-α and TGF-β were measured as pg/g protein.

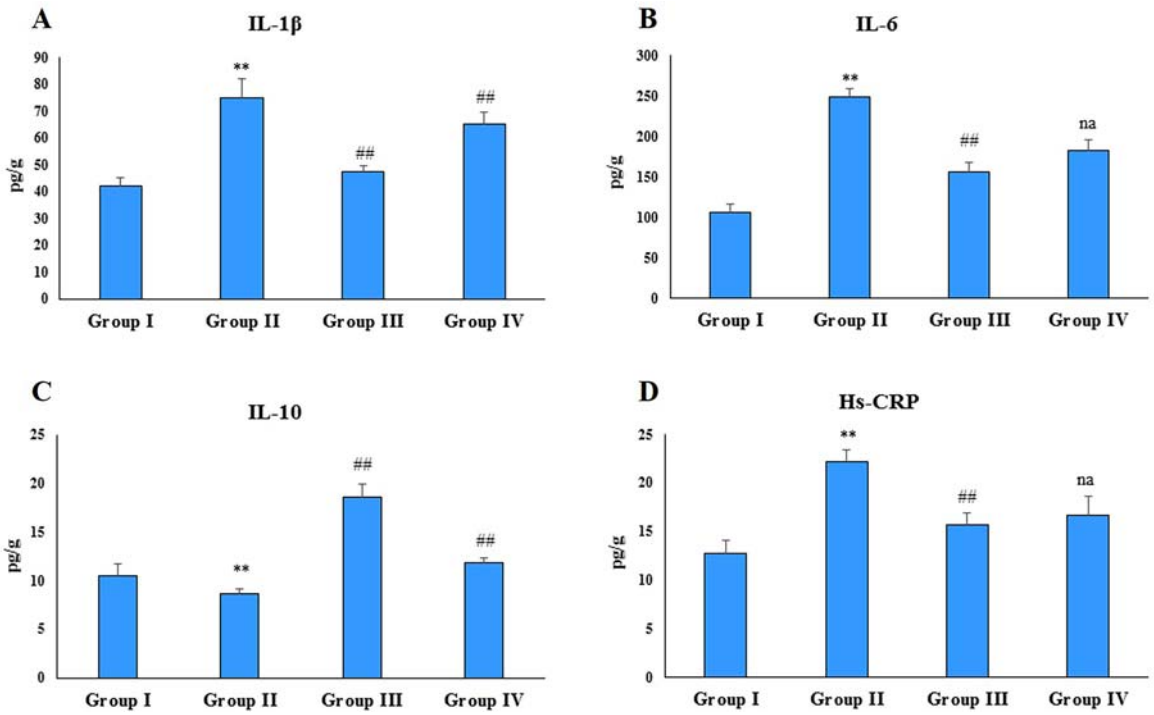

Figure 3. Effect of CFA and zingerone on IL-1β, IL-6, IL-10 and Hs-CRP in cell-free extract of joint. Values are presented as mean ± SE; $n = 6$ animals in each group. **indicates significance at $P < 0.01$ from the control group. ##indicates significance at $P < 0.01$ from the CFA group, na = non-significant. IL-1β, IL-6, IL-10 and Hs-CRP were measured as pg/g protein.

however, group IV animals did not exhibit a significant decrease in these cytokines compared to the disease control. Furthermore, Zingerone treatment restored the levels of IL-10 in both the treatment groups compared to the disease control.

Effect of zingerone and FCA on inflammatory cytokines, TGF-β and Hs-CRP in serum

FCA treatment resulted in a significant ($P < 0.001$, $P < 0.001$ and $P < 0.05$) elevation in the NF-κB, TNF-α and TGF-β levels in group II (Figure 4(A–C)). The administration of zingerone significantly restored the NF-κB, TNF-α and TGF-β levels in the treatment group 1 (group III) compared to the disease control. In the treatment group II (group IV), zingerone administration restored the levels of TNF-α level; however, zingerone treatment had no significant effect on NF-κB and TGF-β levels compared to the disease control.

Administration of FCA also resulted in a significant ($P < 0.01$, $P < 0.001$ and $P < 0.001$) elevation in the serum levels of pro-inflammatory cytokines IL-1β, IL-6 and Hs-CRP in the disease control (Figure 5(A,B,D)) and a significant ($P < 0.001$) decrease in IL-10 compared to the normal control (Figure 5 (C)). Zingerone treatment restored the levels of IL-1β, IL-6, Hs-CRP and IL-10 in the group III compared to the disease control, while as the group IV-treated rats did not show any significant alteration in IL-1β and IL-6 level compared to the group II. However, levels of Hs-CRP and IL-10 were restored significantly in the group IV compared to the group II with zingerone treatment.

Discussion

Arthritis is a group of diseases considered to encompass more than a hundred inflammatory or degenerative conditions. Arthritis not only affects the joints, but also damages other organ systems that may have direct or indirect effect on the joints. Hence, it is essential to decipher the effect of drugs on arthritis by examining pathological and biochemical aspects which are mandatory for evaluating the effect of drugs [40]. Osteoarthritis and rheumatoid arthritis are the most prevalent forms of arthritis resulting in pain, inflammation, mobilization of cells, increase in the tissue size that culminates in loss of defense and joint function [41]. Rheumatoid arthritis is a chronic inflammatory disease that affects nearly 1% population in developed countries [42]. Because of resemblance in symptoms, the adjuvant-induced arthritic rat model is widely used and accepted for assessing and understanding the effect of drugs and various agents in rheumatoid arthritis [43, 44]. The traditional use of plants as medicines by ancient people provides the basis about which part of the plant may be useful for a specific ailment. Also, the increasing demand by consumers in natural therapies and products specifies that compositional characteristics of these products are prerequisite. Since the present treatment regimens available for the rheumatoid arthritis have adverse side effects and are quite expensive, products derived from plants without such disadvantages provide new opportunities. Compounds obtained from plants with a potential to alter or modify the progression of disease clearly have a protective or curative role against rheumatoid arthritis.

In the present study, zingerone was evaluated for its preventive and therapeutic role in FCA-induced rheumatoid arthritis in experimental animals, by mediating with inflammatory and oxidative processes. Improvement in the swelling of paw is the measure of anti-arthritic activity of a number of drugs. The monitoring of swelling in the paw is easy and a reliable method for evaluating the degree of inflammation [45, 46]. As rheumatoid arthritis represents a chronic inflammatory condition, the increased paw edema, as a result of ligament and joint capsule swelling, involves accumulation of granulocytes and monocytes and increased activation of

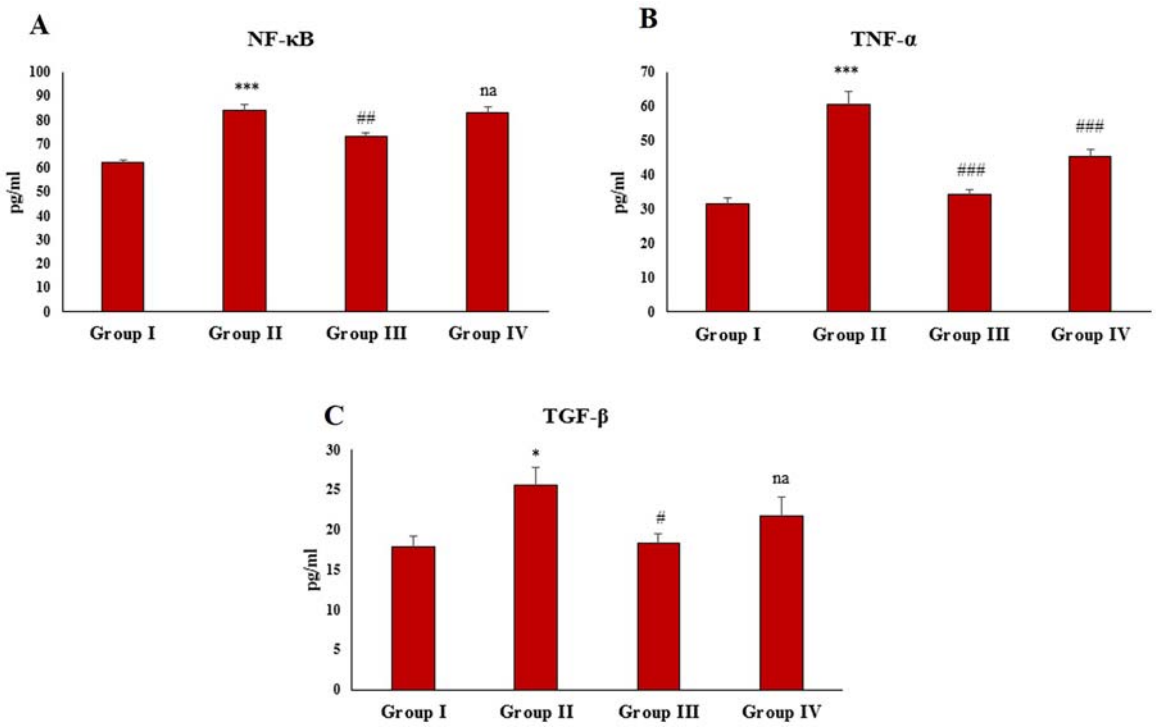

Figure 4. Effect of CFA and zingerone on NF-κB, TNF-α, and TGF-β levels in serum. Values are presented as mean ± SE; $n = 6$ animals in each group. *indicates significance at $P < 0.05$ from the control group. ***indicates significance at $P < 0.001$ from the control group. #indicates significance at $P < 0.05$ from the CFA group. ##indicates significance at $P < 0.01$ from the CFA group. ### indicates significance at $P < 0.001$ from the CFA group. na = non-significant. NF-κB, TNF-α and TGF-β were measured as pg/ml.

macrophages. These macrophages produce several cytokines, such as IL-6 and TNF-α linked with rheumatoid arthritis [47]. In the present study, zingerone treatment demonstrated anti-arthritic potential. The elevation in the thickness of paw after intra-dermal immunization with FCA is the indicator of rheumatoid arthritis. The treatment with zingerone showed decrease in the thickness of paw and in the diameter of joint by interfering with the inflammatory mediators, indicating its anti-inflammatory ability in FCA-induced rheumatoid arthritis.

NF-κB is the key player for initiating and intensifying inflammation in rheumatoid arthritis [48, 49]. NF-κB along with its inhibitory-kB (IκB) is located in the cytoplasm complex. On activation, inhibitory-κB kinase (IKK) through

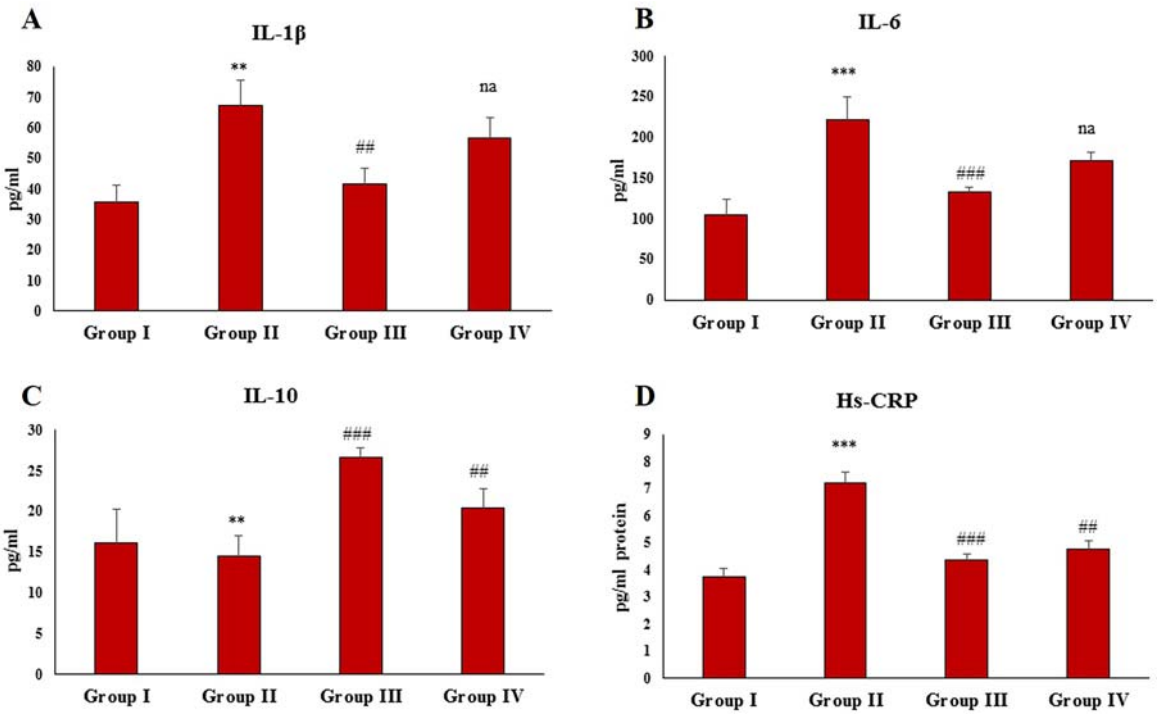

Figure 5. Effect of CFA and zingerone on IL-β, IL-6, IL-10 and Hs-CRP levels in serum. Values are presented as mean ± SE; $n = 6$ animals in each group. **indicates significance at $P < 0.01$ from the control group. ***indicates significance at $P < 0.001$ from the control group. ##indicates significance at $P < 0.01$ the CFA group. ### indicates significance at $P < 0.001$ from the CFA group. na = non-significant. IL-1β, IL-6, IL-10 and Hs-CRP were measured as pg/g protein.

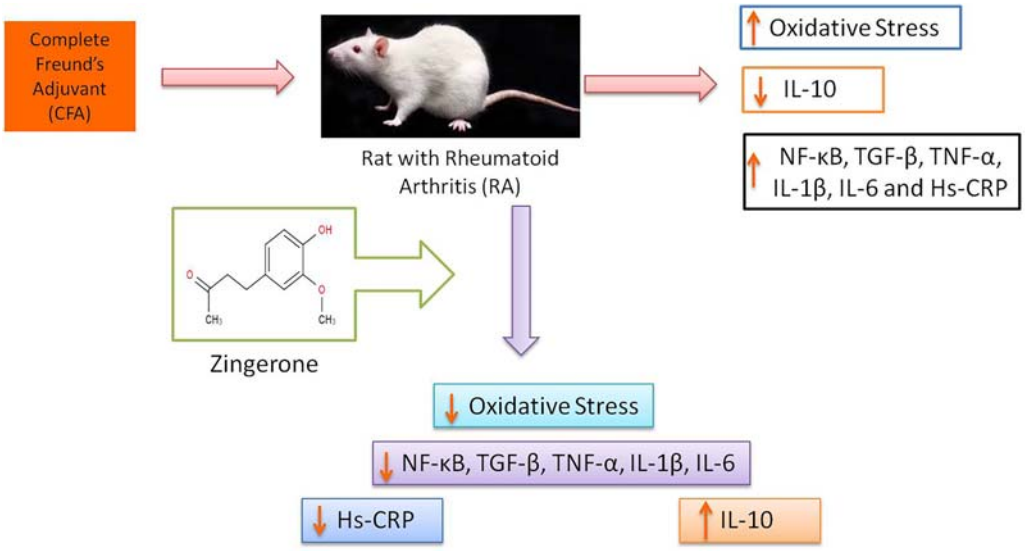

Figure 6. Different mechanism of action of zingerone in arthritis.

phosphorylation degrades IκB and activates NF-κB. The activated, NF-κB gets transferred to nucleus and triggers the synthesis of inflammatory mediators, TNF-α and iNOS. Therefore, regulation of NF-κB activity by blocking its transfer to the nucleus or through inhibition of its binding to DNA can be employed for the modulation of inflammation and cellular injury. A number of studies have revealed that zingerone attenuates NF-κB activation that results in the reduction of cell damage [30]. Thus, this study was designed to examine the protective effect of zingerone on NF-κB activity in FCA-induced rheumatoid arthritis. In agreement with the stated arguments, in the present study, FCA inoculation resulted in elevated levels of NF-κB, which was corrected by zingerone. This role of zingerone in reducing the joint inflammation may be either due to its ability to prevent the transfer of NF-κB to the nucleus or inhibiting its binding to DNA. Moreover, NF-κB regulates innate and adaptive immunity by the activation of inflammatory cytokines such as, TNF-α, IL-1β, IL-6 and enzyme nitric oxide synthase and COX. Activation of NF-κB worsens the rheumatoid arthritis [50]. Tumor necrosis factor alpha (TNF-α), in turn, stimulates production of other inflammatory markers, such as IL-1β and IL-6, which further facilitates infiltration of leukocytes and vasodilatation at the site of disease [51]. In addition, these inflammatory cytokines mobilize neutrophils and monocytes toward the joint through activation of chemokines [52]. To prevent the destruction of bone and cartilage, it is essential to inhibit TNF-α responsible for the activation of matrix metalloproteinase (MMPs) [53]. The present study resulted in significant reduction in the levels of TNF-α, IL-1β and IL-6 in contrast to the diseased control group. So our findings are in concurrence with the results of Rehman et al. [54] that zingerone treatment significantly prevents NF-κB activation and decreased levels of TNF-α, IL-6 and IL-1β in an experimental model of type 2 diabetes.

IL-10 is the immune-regulatory cytokine, which impedes inflammation and the damage of bone and cartilage during rheumatoid arthritis by suppressing the activation of NF-κB through inhibition of IKK [55]. IL-10 not only improves the integrity of joint during rheumatoid arthritis, but also inhibits T helper cell-activated cytokines mostly TNF-α and IL-1 [56]. The findings of the current study revealed a significant increase in the IL-10 levels in the zingerone treatment group compared to the disease control group.

TGF-β acts as a regulatory anti-inflammatory cytokine; however, in some conditions it may work as a pro-inflammatory molecule [57, 58]. In rheumatoid arthritis, TGF-β has been reported to act as a pro-inflammatory cytokine with increased levels in plasma and synovial fluid [59, 60]. The increase of TGF-β in the arthritic untreated group compared to the normal control group and the decreased level with the administration of zingerone indicates that it acts as a pro-inflammatory molecule in rheumatoid arthritis. So, our findings are in concurrence with the above findings. Moreover, serum CRP is the biomarker of systemic inflammation, which represents active inflammation. Increase in CRP is the reflex of severity and progression of arthritis [61]. Increase in the levels of IL-6 and TNF-α further alleviates the levels of CRP as reported by Kumar et al. [31]. In the present study, FCA resulted in an increase in CRP levels and treatment with zingerone not only reduced the FCA-induced CRP changes but also reduced inflammation, as indicated by a low level of CRP, similar observations have been reported by Kalaiselvan & Rasool [62].

Oxidative stress adds to the progression of rheumatoid arthritis. Increased production of ROS causes damage to joints by the activation of matrixmetallo-proteinases and osteoclast activity [63]. Reactive oxygen species (ROS) above thresh hold levels not only activate nuclear factor kappa-β (NF-κB) and pro-inflammatory cytokines but also damage the lipids, proteins, membranes, nucleic acids [64] and precipitate destruction of the joint tissue [65]. Peroxidation of lipids could contribute to cell damage by altering the properties of biological membranes and plays an essential role in the progress of disease. Additionally, certain products of lipid peroxidation have been reported to activate genetic over-expression of proteins such as cytokines [66]. Zingerone is reported to prevent LPO and to possess SOD like activity [67]. Similarly, in the present study, higher amounts of liver and joint MDA or lipid peroxides were observed in rats immunized with FCA which was significantly decreased with zingerone treatment, findings are in concurrence with Ahmad et al. [58] and Oboh et al. [68].

As reported previously, oxygen-free radicals result in oxidative stress and elevation in GPx and catalase activity in

rheumatoid arthritis-induced rats, compared to the control. This imbalance in the activity of enzymatic antioxidants is corrected with zingerone treatment, thereby indicating antioxidant activity of zingerone in rheumatoid arthritis with decrease in the production of ROS and hence preventing the tissue damage by oxidative stress. These findings are in concurrence with the previous reports of Hemalatha et al. [69]; Alvana et al. [70], Rao and Rao [71] and Rahmani et al. [72] and Saleem et al. [73]. The significant reduction in LPO and elevation in antioxidants (SOD, catalase and GPx), in arthritic rats that received zingerone from the day first of rheumatoid arthritis induction, emphasizes the role of zingerone in protecting the organ damage and bone loss in rheumatoid arthritis rat model by scavenging the free radicals.

Conclusion

As manifested from the results of the present investigation, zingerone supplementation reversed the FCA-induced changes by reducing lipid peroxidation, increasing the activities of enzymic antioxidants, and regulating the levels of pro-inflammatory cytokines and the inflammatory mediators. These biochemical and molecular findings reveal the potent antioxidant and anti-inflammatory properties of zingerone against FCA-induced rheumatoid arthritis, which suggests that the protective effect of zingerone probably might be through the attenuation of oxidative stress and inflammation (Figure 6). Thus, on the basis of our findings, the combined antioxidant and anti-inflammatory properties of zingerone can prove to be a remedial measure for the prevention and treatment of arthritic joint diseases.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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