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## RESEARCH ARTICLE

### TNF ALPHA, IFN GAMMA AND CATALASE LEVELS IN OSTEOARTHRITIS: CONTROL AND REGULATION BY PHYTOCONSTITUENTS

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#### ABSTRACT

Tumor necrosis factor (TNF)-alpha is a pro-inflammatory cytokine secreted by monocytes/macrophages, T cells, and natural killer (NK) cells. Interferon gamma (IFN- $\gamma$ ) is an acid-labile interferon produced by CD4 and CD8 T lymphocytes as well as activated NK cells. IFN- $\gamma$  receptors are present in most immune cells, which respond to IFN- $\gamma$  signaling by increasing the surface expression of class I MHC proteins. This promotes the presentation of antigen to T-helper (CD4+) cells the increased presence of TNF-alpha and its receptors in articular cartilage with mild osteoarthritic. Regulating TNF-alpha is an important component in the treatment. Osteoarthritis (OA) is an inflammatory joint disease due to degeneration of joint cartilage and underlying bone. Inflammation is common and essential protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, and physical agents, and ischemia. The main characteristics of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as arthritis, chronic asthma, multiple sclerosis, inflammatory bowel disease. Osteoarthritis is characterized by loss of joint cartilage, results pain and loss of function primarily in the knees and hips, which mostly affects 9.6% of men and 18% of women aged more than 60 years. The present sequel was emphasized on the levels of TNF alpha, IFN gamma and catalase in osteoarthritic patients and to compare it with the levels of those after introducing of antioxidants (Phytoconstituents). Control and regulation through phytoconstituents was enhanced a new way to prevent Osteoarthritis. Study have explored and examined on several medicinal plants spices like Curcumin (*Curcuma longa*), Cardamom ( *Elettaria cardamom*), Saffron (*Crocus sativus*), Cinnamon (*Cinnamomum verum*) which are carotenoid-rich that is used to treat various types of diseases caused by oxidative stress specially for Osteoarthritis, Diabetics etc. It has the ability to decrease the levels of ROS, free radical-mediated lipid peroxidation, its anti-inflammatory effects which stimulate phosphorylation of AMP-activated protein kinases (AMPK), acetyl-CoA carbohydrate (ACC), and mitogenactivated protein kinases (MAPKS). Cinnamon consists of cinnamaldehyde, cinnomic acid, eugenol and other components that have antioxidant and anti-inflammatory effects that can reduce interleukin (IL) 6, and nitric oxide (NO) types of production and can suppress lipid peroxidation and malondialdehyde (MDA). Hence, regulation and control of Osteoarthritis is a challenging opportunity at present 21st century, enhancement of ushering a new way to evaluate and prevent Osteoarthritis among the Indian patients can possible through such medicinal herbs which were accentuated upon.

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## INTRODUCTION

Tumor necrosis factor (TNF)-alpha is a pro-inflammatory cytokine secreted by monocytes/macrophages, T cells, and natural killer (NK) cells. TNF-alpha is synthesized as a 233 amino acid (aa) transmembrane protein (mTNF-alpha) with a theoretical molecular weight (MW) of 26 kDa as well as a 157 aa, 17 kDa soluble protein (sTNF-alpha). Interferon gamma (IFN- $\gamma$ ) is an acid-labile interferon produced by CD4 and CD8 T lymphocytes as well as activated NK cells. IFN- $\gamma$  receptors are present in most immune cells, which respond to IFN- $\gamma$  signaling by increasing the surface expression of class I MHC proteins.

This promotes the presentation of antigen to T-helper (CD4+) cells. IFN- $\gamma$  signaling in antigen-presenting cells and antigen-recognizing B and T lymphocytes regulate the antigen-specific phases of the immune response. Additionally, IFN- $\gamma$  stimulates a number of lymphoid cell functions including the anti-microbial and anti-tumor responses of macrophages, NK cells, and neutrophils. Human IFN- $\gamma$  species-specific and is biologically active only in human and primate cells. Recombinant human IFN- $\gamma$  is a 16.7 kDa protein containing 143 amino acid residues. The increased presence of TNF-alpha and its receptors in articular cartilage with mild osteoarthritic changes suggests a role in the development of early OA (Kammermann & et. Al 1996).

Regulating TNF-alpha may be an important component in the treatment of OA. Osteoarthritis (OA) is an inflammatory joint disease due to degeneration of joint cartilage and underlying bone. Inflammation is common and essential protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, and physical agents, and ischemia. The main characteristics of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as arthritis, chronic asthma, multiple sclerosis, inflammatory bowel disease. Osteoarthritis is characterized by loss of joint cartilage, results pain and loss of function primarily in the knees and hips, which mostly affects 9.6% of men and 18% of women aged more than 60 years (1)

**Causes and Consequences:** Osteoarthritis includes four important phases: The initial phase, the inflammatory phase, pain and degradation phase. The inflammatory phase results by increased pro-inflammatory cytokines and leukocyte numbers in the affected joints. Interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) activates the degradation enzymes such as metalloproteinase, collagenase, gelatinase and aggrecanase which aggravates the inflammation in the joints affected by OA (2). Inflammation can induce bone damage and that occurs by two processes which are linked via common mediators. These mediators are receptor activator of NF- $\kappa$ B ligand (RANKL) and its receptor RANK, proinflammatory cytokines (e.g., tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 1 (IL-1), IL-6, IL-17, and IL-18), and matrix-degrading enzymes (e.g., matrix metalloproteinases (MMPs) and cathepsin K (1)). In OA tissue other pro-inflammatory cytokines, including IL-8, LIF, IL-6, IL-11, and IL-17, are observed to be over expressed, and therefore be considered potential contributing factors in the pathogenesis of this disease. Two of them, IL-11 and IL-6, have also shown anti-inflammatory properties (3).

Damage-associated molecular pattern (DAMP) is generated by damaging of cellular and cartilage extracellular matrix (ECM) products from trauma or normal aging or from repetitive overuse which can activate the innate immune system (4) (5). DAMP can be fragments, that are generated from proteins, proteoglycans, or remnants of cellular breakdown as uric acid. (6) A sterile inflammatory response through interaction with particle recognition receptors (PRR) is elicited by DAMP, PRR such as Toll-like receptors (TLR), on the surface of immune cells, or with PRR in the cell cytoplasm, such as NOD-like receptors (4)(5)(7). The migratory cells such as phagocytic cells, macrophages play a vital role in host defense system, which are an important source of cytokines that help to activate T cells and B cells (8). Among the cytokine families, the interleukin (IL)-1 family is closely linked to the innate immune response. The cytoplasmic domain of the IL-1 receptor type I is homologous to the cytoplasmic domains of all Toll-like receptors (TLRs). Thus, fundamental inflammatory responses including the induction of cyclooxygenase type 2, increased expression of adhesion molecules, or synthesis of nitric oxide are identical responses of both IL-1 and TLR ligands. The antigen recognition and lymphocyte function are affected by both families. As IL-1 $\beta$  plays a salient role in mediating auto-inflammatory diseases, it is the most studied member of the IL-1 family. (9) Although the TLR and IL-1 families evolved to assist in host defense against infection, unlike the TLR family, the IL-1 family also includes members that suppress inflammation, both specifically within the IL-1 family but also nonspecifically for TLR ligands and the innate immune response. Activation of TLR leads to increased expression of pro-inflammatory cytokines through a number of transcription factors. The factors are activator protein 1, cyclic AMP responsive element binding protein, interferon regulatory factors, and nuclear factor- $\kappa$ B (NF- $\kappa$ B), that latter play a role in OA (6). Analgesia and anti-inflammatory drugs, including steroids can able to suppress the symptoms of inflammatory diseases. Disease-modifying antirheumatic drugs (DMARDs), anti-tumour necrosis factor (TNF)- $\alpha$  therapy (infliximab), anti-CD20 therapy and corticosteroids methylprednisolone which are newer therapies, are used to inhibit the immune process but these agents are associated with numerous side effects. Hence, a safer anti-inflammatory drug for such conditions which does not have side effects is needed (1). The

oxidative stress is produced by free radicals mainly reactive oxygen species (ROS). Oxidative stress causes tissue damage. Free radicals are the reason of diverse diseases and disorders like diabetes and related problems of ageing. In the form of natural antioxidants medicinal plants can act as major defense against free radicals. Antioxidants are those substances that play an important role in neutralizing such free radicals (1). Free radicals play a dual role in our body, deleterious as well as beneficial species. If the concentration of free radical is moderate, it involves in normal physiological functions but oxidative stress occurs by excess production of free radicals or decreased level of antioxidants. It is a harmful process which can rupture or damage the cell structures, including lipids, proteins, RNA and DNA that leads to numerous diseases. Synthetic medicines play a role to generate free radicals in body which are employed in the treatment of different diseases (10). ROS can damage the cell structures, nucleic acids, lipids and proteins at high concentration. Lipid peroxidation and decreased antioxidant defense system enzyme such as catalase (CAT) and glutathione peroxidase (GPx) can occur due to O<sub>2</sub> which can damage to the ribonucleotide that is required for DNA synthesis (10).

The main source of antioxidants, phyto-constituents are plants, which may be capable to minimize the free radical reactions and prevent our body from oxidative stress related damage. Vegetables and fruits are also rich in antioxidant substances which can reduce free radical activities to prevent oxidative stress. There is a great demand of modern era to use phyto constituents or phyto medicines to prevent oxidative stress that cause tissue injury than synthetic medicine as these don't have any known side effects. (10) Some medicinal plants or herbs play important role in treatment of osteoarthritis such as ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), green pepper (*Capsicum annum*), sesame (*Seasmum indicum*), garlic (*Allium sativum*), nutmeg (*Myristica fragrams*), Clove (*Syzygium aromaticum*). There are different parts of medicinal plant can be used to reduce the pain of osteoarthritis. Such as plant bulb of garlic (*Allium sativum*), rhizomes of turmeric (*Curcuma longa*), dried flower buds of clove (*Syzygium aromaticum*), seed leaves of nutmeg (*Myristica fragram*), seeds of sesame (*Seasmum indicum*), fruit of citron (*Citrus medica*), rhizome of ginger (*Zingiber officinale*), leaves and barks of blue gum (*Eucalyptus globulus*), leaves and flowers of common lavender (*Lavandula officinalis*), leaves of pudina (*Mentha arvensis*), fresh or dried flower heads of mountain amica (*Arnica montana*), flower of marigold (*Calendula officinalis*). Among these ginger contains an anti-inflammatory chemical substance which can inhibit cyclooxygenase and 5-lipoxygenase. (11) Sesame (*Sesamum indicum* L.) is a traditional health food which has been used to improve nutritional status and prevent various diseases. The seeds are rich in oil, protein and lignans that may have antioxidant and hypo-cholesterolemic effects. Sesame seeds are used to evaluate lipid profile and oxidative stress in OA patients. (12) Garlic (*Allium sativum*; Liliaceae) is one of important Indian traditional medicine that has medicinal value and has been used in the treatment of several diseases. Pharmacologically, garlic acts as hypolipidemic, anticoagulant and anticancer. It may be capable to modulate oxidative stress which plays the major role in osteoarthritis. (1) Now a days Arnica Montana Lis most frequently used for the treatment for symptomatic relief in OA as it has an anti-inflammatory action and it may can inhibit the transcription factor NF- $\kappa$ B, a central mediator in inflammatory process that controls the transcription of various cytokine genes (13) Curcumin (*Curcuma longa*) was investigated mainly for its anti-inflammatory and anti-oxidant potency. As oxidative stress damages tissues that can cause osteoarthritis thus, curcumin or turmeric can be used as anti-inflammatory substance. Curcumin may inhibit the activation of NF- $\kappa$ B and translocation induced by IL-1 $\beta$  (14).

**Objective:** To investigate the levels of TNF alpha, IFN gamma and Catalase in osteoarthritis and its control and regulation by certain phytoconstituents. The aim of this study was to evaluate the levels of TNF alpha, IFN gamma and catalase in osteoarthritic patients and to compare it with the levels of those after introducing of antioxidants (Phytoconstituents).

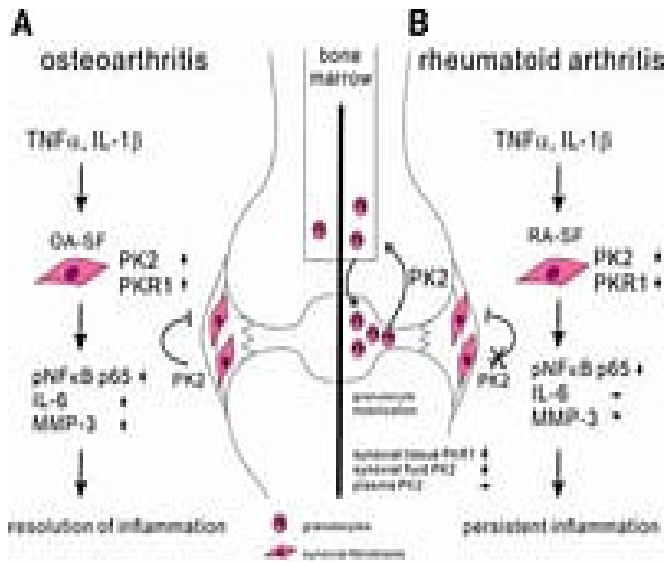


Fig of Osteoarthritis

To investigate the diagnosis of osteoarthritis depending on Patient history, Radiological examination and findings,

To examine anatomical examination and escalate findings,  
 To determine clinical examination, laboratory tests examine through biochemical and immunological data.  
 To measure Catalase, TNF alpha and IFN gamma activities.

**METHODOLOGY**

**Experiment procedure:** The experiment was designed based on diagnosis of osteoarthritis depending on

- Patient history
- Radiological examination and finding
- Anatomical examination and finding
- Clinical examination and finding
- Laboratory tests having biochemical and immunological data

**Participants:** The data were taken to study the antiarthritic activity of some phytoconstituents from 20 either male or female blood serum samples of above 40 years old. Participants had pain complain, which can be resulting from inflammation of synovial cavity or degeneration of articular cartilage. A data from approximately normal human sample of same aged was taken as control.

**Participants' health status:** There was no nutritional deficiency of the participants except calcium deficiency as they were all aged. Among them mostly were female.

**Laboratory tests having biochemical and immunological data**

- Catalase by spectral assay
- TNF alpha by ELISA
- IFN gamma by ELISA

**RESULTS**

The result includes three categories of determinations. Catalase activity by spectral assay, TNF alpha activity by ELISA and IFN gamma activity by ELISA method

**Catalase by spectral assay:** Catalase activities were measured from three patients. It is seen from the bar diagrams that at the start of the treatment because of oxidative stress catalase activities were very high which is gradually decreases between 21 to 42 day of treatment.

Patient-1

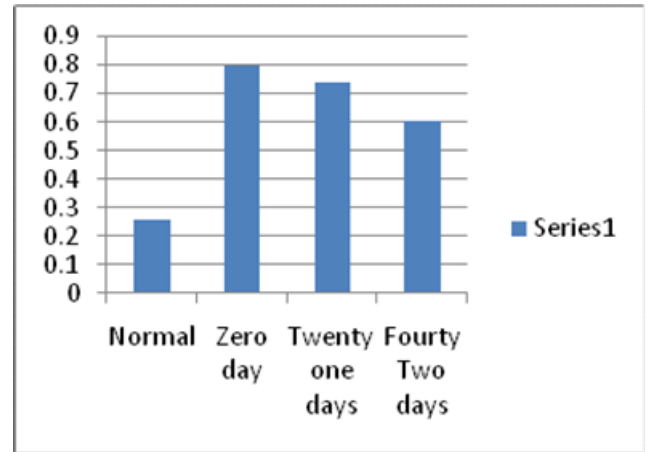


Fig:1.1 Bar diagram showing the catalase activity of patient-1 and it can be seen that particular phytomedicine is working after twenty one days and forty two days, which was determined by spectral assay method

Patient-2

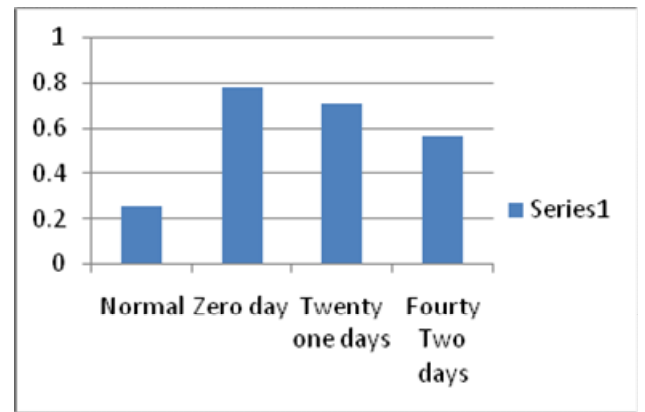


Fig. 1.2 1 Bar diagram showing the catalase levels of patient-2 and it can be seen that particular phytomedicine is working after twenty one days and forty two days

Patient-3

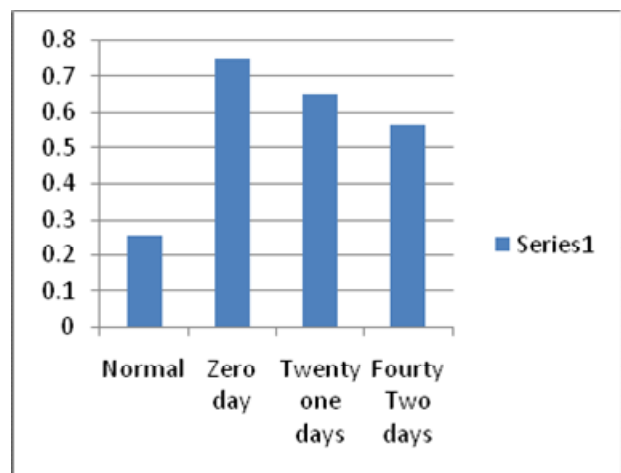


Fig. 1.31 Bar diagram showing the catalase levels of patient-3 and it can be seen that particular phytomedicine is working after twenty one days and forty two days

**TNF alpha by ELISA:** TNF alpha, which is a proinflammatory cytokines is increased at the beginning of the treatment in all the four patients, which is significantly decreased between 21 to 42 day of treatment almost to normal value

Patient-1

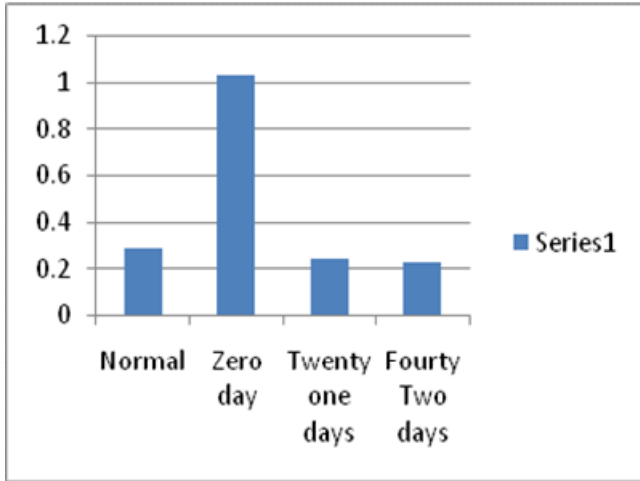


Fig. 2.11 Bar diagram showing the TNF alpha levels of patient-1 and it can be seen effectiveness of the particular phytomedicine on the zero day, after twenty one days and forty two days by ELISA method

Patient-2

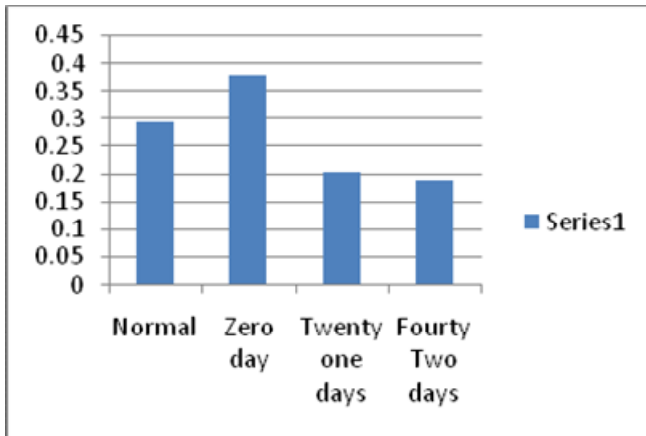


Fig. 2.21 Bar diagram showing the TNF alpha levels of patient-2 and it can be seen effectiveness of the particular phytomedicine on the zero day, after twenty one days and forty two days

Patient-3

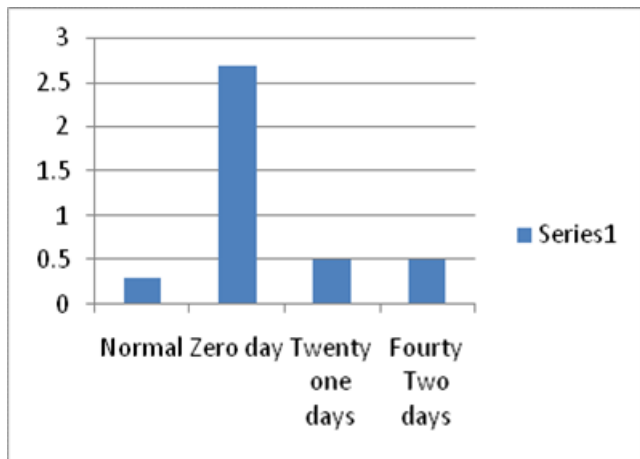


Fig. 2.31 Bar diagram showing the TNF alpha levels of patient-3 and it can be seen that particular phytomedicine is working after twenty one days and forty two days

Patient-4

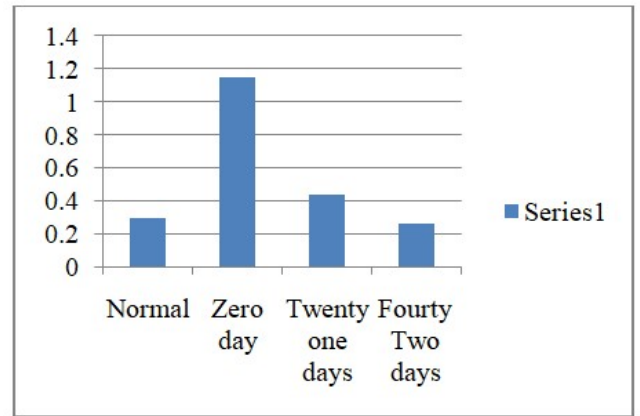


Fig. 2.41 Bar diagram points out the TNF alpha levels of patient-4 and the activity of phytomedicine on the zero day, after twenty one days and forty two days can be seen.

IFN gamma by ELISA

IFN gamma similar trends of results are also observed so far the activity of IFN gamma is concerned

Patient-1

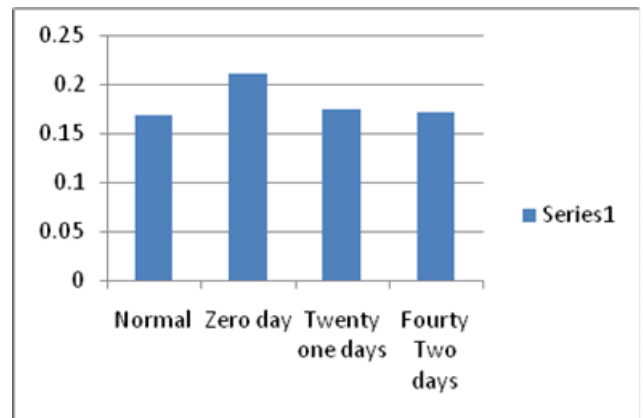


Fig. 3.11 Bar diagram exhibits the IFN gamma levels of patient-1 and it can be seen that particular phytomedicine is working after twenty one days and forty two days

Patient-2

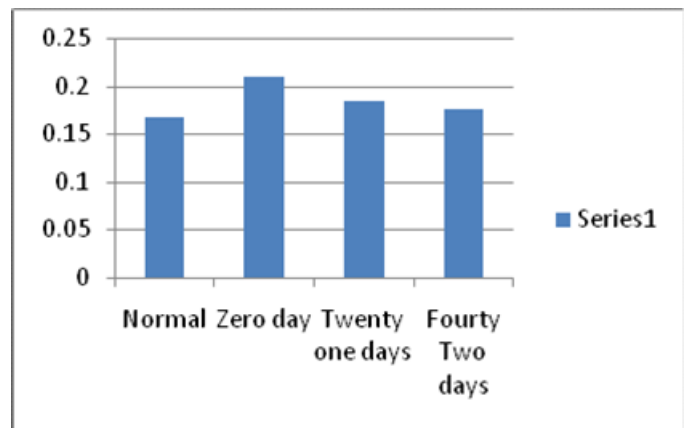


Fig. 3.21 Bar diagram showing the IFN gamma levels of patient-2 and it can be seen that particular phytomedicine is working after twenty one days and forty two days



## Patient-3

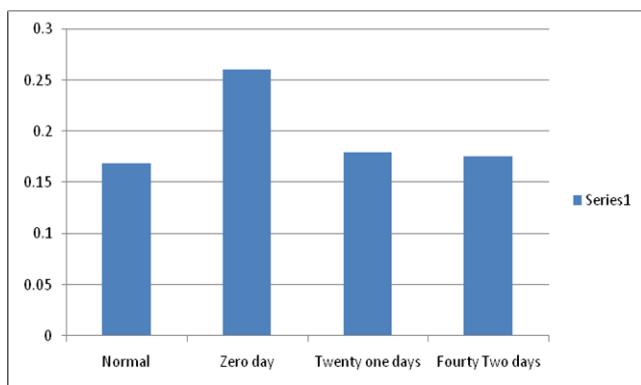


Fig. 3.31 Bar diagram denotes the IFN gamma levels of patient-3 it can be seen that particular phytomedicine is working after twenty one days and forty two days.

## DISCUSSION

With the production of pro-inflammatory cytokines, chronic inflammatory changes are seen in the patients with OA. The introduction of drugs like aspirin, cortisone and others has totally changed the treatment of rheumatic and musculoskeletal disorders. The efficacy of cortisone and other painkillers in these inflammatory disorders has stopped the way for introducing newer anti-inflammatory agents. Although these steroidal and non-steroidal drugs have brought a revolutionary change for inflammation in safety factor these drugs could not give a perfect satisfaction as these have so many side effects, so a safer anti-inflammatory drug is needed treat various arthritic, rheumatic conditions. There are several plants, which is having medicinal value in there different body parts and accordingly some of these medicinal plants have been investigated for anti-inflammatory and other pharmacological properties. Oxidative stress caused by osteoarthritis can be reduced by decreasing the level of inflammatory mediators such as TNFs, cytokines. According to various reports TNF produces from macrophages which are activated by several stimuli. These production of TNF can be suppressed by curcumin.

Numerous reports have suggested that TNF alpha in macrophages and monocytes can be induced by LPS. And the expression of TNF alpha can be down regulated by curcumin. Various reports have suggested that curcumin has the ability to suppress the production of TNF that is expressed by microglial cells, myeloid cells, adipocytes and other cell types. (15) Numerous studies have concluded that curcumin can down-regulate the expression of TNF- $\alpha$  by the synthesis of LPS which is one of the major inducers of TNF- $\alpha$  in macrophages and monocytes (16). Apart from curcumin, cardamom is a herb which contains sterol and phenolic acids (essential lipids). These have antioxidative potential which might increase the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.

It is suggested that the production of nitric oxide by macrophages might be reduced by cardamom that exerts anti-inflammatory effects. Studies have concluded that saffron, a spice which is carotenoid-rich that is used to treat various types of diseases caused by oxidative stress specially for diabetes (17). It has the ability to decrease the levels of ROS, free radical-mediated lipid peroxidation (18). It has anti-inflammatory effects and might stimulate phosphorylation of AMP-activated protein kinases (AMPK), acetyl-CoA carboxylase (ACC), and mitogen activated protein kinases (MAPKs) (17). Cinnamon consists of cinnamaldehyde, cinnamic acid, eugenol and other components that have antioxidant and anti-inflammatory effects that can reduce interleukin (IL) 6, and nitric oxide (NO) types of production and can suppress lipid peroxidation and malondialdehyde (MDA) (17).

## CONCLUSION

Osteoarthritis is a degenerative disease which can damage the joint cartilages. There are different factors which can induce the level of these degradation such as increased amount of catalase, TNF alpha and IFN gamma which can be controlled by disease-modifying anti-rheumatic drugs (DMARDs) but these have numerous side effects. Various reports have suggested that there are several medicinal plants, herbs, spices which have an antioxidant properties in them which can act to reduce the oxidative stress as well as the levels of catalase, TNF alpha and IFN gamma. Among these Curcumin (*Curcuma longa*), cardamom, saffron are some spices which are very common in day to day diet that might have the capacity to decrease the levels of oxidative stress which can cause osteoarthritis and such diseases.

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