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Mechanisms of action of native collagen type II and Aflapin® on the pathophysiology of osteoarthritis and their evidences

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ABSTRACT

Osteoarthritis (OA) is a degenerative and chronic inflammatory disease that affects the entire joint tissue such as articular cartilage, synovial membrane, subchondral bone, and ligaments. Imbalance between anabolism and catabolism lead to degradation of articular cartilage which may further initiate inflammatory cascades. There is an interplay of mechanical and immune-mediated injuries that lead to cartilage destruction and inflammation in OA. The mainstay of OA treatment involves drugs like paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) which provide symptomatic relief in many cases; but are unable to inhibit disease progression. Also, long-term use of these drugs, is associated with major safety concerns. Native collagen type II (NC-II) and Aflapin, especially when used in combination, can slow down the disease progression in OA and serve as a safer and effective treatment option for OA. NC-II is non-hydrolyzed collagen having intact triple helix structure with active epitopes and antigenicity. Aflapin is a novel synergistic composition of Boswellia serrata gum resin having higher composition of 3-O-acetyl-11-keto-betaboswellic acid (AKBA). Various experimental studies have demonstrated the mechanisms by which both these agents exert their benefit in OA. Further, multiple clinical studies have demonstrated the efficacy and safety of NC-II and Aflapin, when used individually or as a combination. This is a narrative review of the pathophysiology of OA, current treatment modalities, and non-clinical and clinical evidence of the beneficial effects of NC-II and Aflapin in the management of OA.

Keywords: Undenatured collagen, Boswellia serrata extract, Oral tolerance, Cartilage regeneration

INTRODUCTION

Osteoarthritis (OA) is a degenerative and chronic inflammatory disease that affects the entire joint tissue such as articular cartilage, synovial membrane, subchondral bone, and ligaments.[1,2] OA mostly causes damage to weight bearing joints like knee, hip and hands by repeated and long-term wear and tear of the cartilage tissue.[3] Being an inflammatory disease, synovial membrane of OA patients was frequently observed with the presence of mononuclear cell infiltrates consisting of T cells and macrophages. Also increased levels of immunoglobulins and number of mononuclear cells were exist in synovial fluid of OA patients.[1,4] Clinical manifestations of OA such as swelling, joint pain, muscle weakness, stiffness and restricted movement may lead to decreased productivity and quality of life of patients.

Although the exact cause of OA is difficult to capture, a list of risk factors can be considered for the development of OA, including traumatic injury, obesity, occupation, genetic predisposition, hormonal disorders, mechanical stress, age gender, diet, and inflammation caused by infection or surgery.[5,6] OA is a functional disorder leading to impaired quality of life. The disease is mostly associated with elderly people since more than 50% of people above 60 years of age get affected by OA. About 15% of people worldwide suffer from OA which increases global socioeconomic burden whereas in India also as per a community based cross sectional study, 28.7% is the prevalence of knee OA. It was observed that female population is more prone to OA as compared with men and risk of disease increases with the increasing age.[7]

PATHOPHYSIOLOGY

OA pathophysiology is mainly characterized by degradation of articular cartilage, which occurs because of imbalance between anabolism and catabolism process. The major structural component of cartilage is type II collagen, which in association with aggrecan and proteoglycans forms the network structure of extracellular matrix (ECM). [6-8]

Recent studies showed that the interplay between mechanical and immune pathways has an important role in the pathophysiology of OA. Mechanical injuries like tissue damage, joint overuse, mechanical trauma, etc. initiate the production of destructive enzymes, chemokines and inflammatory cytokines which trigger the mechanical pathway. [1] Along with mechanical injuries, some other risk factors like age, obesity and inflammation may cause increased expression of chondrocytes' catabolic factor matrix metalloproteinase-13 (MMP-13). By breaking the peptide bond, MMP-13 cleaves type II collagen leading to cartilage breakdown and inflammation. The cartilage breakdown products from synovial fluid further release cytokines, like interleukin-1β (IL-1β), IL-6, tumor necrosis factor- α (TNF-α), prostaglandin-E2 (PGE2) and MMP-13 which inhibit proteoglycan synthesis and again become responsible for accelerating OA and inflammation.[6,9] Proinflammatory cytokines TNF-α and IL-1β play an important role in inflammatory cascade. These cytokines promote matrix degradation by the production of MMPs and by inhibiting the type II collagen and proteoglycans synthesis in chondrocytes.[1]

Immune pathway in OA pathology may involve cellular immunity in which endogenous articular cartilage components act as antigenic peptides. ECM protects the unique surface antigens present on chondrocytes. Due to the chronic degradation of articular cartilage, these surface antigens of chondrocytes lose the ECM protection, which may activate autoimmune reactions.[1] In the synovial membrane of OA patients, there is a presence of cell infiltration containing macrophages and T cells.[4] When chondrocyte comes in direct contact with T cells, it shows potential to act as an antigen presenting cell (APC). Chondrocyte cells can process exogenous antigens as well as phagocytize collagen fragments. The higher number of interferon-γ (IFNγ)-positive cells (Th1) in the synovium of OA patients indicates a Th1 cell-mediated specific immune response that is driven by local antigens.[1] Th1-mediated IFN-γ production can stimulate the macrophage polarization to M1 (pro-inflammatory). This M1 polarization is associated with high production of pro-inflammatory cytokines and chemokines like TNF-α, IL-1b, IL-6, nitric oxide (NO), MMP-13, PGE2, which further leads to inflammation and cartilage degradation.[4,10] There is also a decrease in action of T regulatory cell and anti-inflammatory cytokine IL10 and transforming growth factor β (TGF β) reduction, leading to promotion of T regulatory differentiation and suppression of MMPs.[11] TGF-β and Insulin-like growth factor-1 (IGF-1) have an important role in the repair of the cartilage. IGF-1 inhibits MMP-13 production via extracellular signal-regulated kinase (ERK) pathway.[6]

Overexpression of cyclooxygenase 2 (COX-2) by OA chondrocytes further leads to an overproduction of PGE2. PGE2 is involved in cartilage degradation by binding to different prostaglandin receptors.[1] Nitric oxide acts as a catabolic factor since it activates MMPs and inhibits collagen and aggrecan synthesis. It may induce chondrocyte apoptosis by increasing susceptibility of chondrocytes to injury by other oxidants.[4]

Cartilage destruction releases phospholipids which get converted to arachidonic acid by phospholipases. Arachidonic acid acts as a substrate for 5-lipoxygenase (5-LOX) and produces leukotrienes (LTs). In osteoblasts, LTs enhance the production of pro-inflammatory cytokines TNF- α and IL-1 which further leads to the production of degrading enzymes like MMPs. LTs also activate chemotactic agents like interstitial cell adhesion molecules (ICAMs) which stimulate migration and activation of T cells and macrophages, inducing phagocytic activity and release of reactive oxygen species (ROS). This ultimately leads to an imbalance between the anabolic and catabolic pathways.[5,11] The combined interplay of both the mechanical and the immune pathways lead to cartilage destruction and inflammation in OA.

CURRENT MANAGEMENT

The mainstay of OA treatment is pain reduction, minimization of joint damage and improved quality of life with functional mobility. Some non-pharmacological treatments such as exercise, weight reduction, physiotherapy, heat/cold therapy, and joint protection can be helpful for maintaining functional mobility of OA patients. Few pharmacological drugs like paracetamol or NSAIDs are also commonly used to treat the condition. It has been observed that these drugs are very useful in symptomatic relief in OA but are not able to repair cartilage destruction or to inhibit disease progression. Also, long-term use of these drugs, as is expected in an OA patient, is associated with major safety concerns such as

gastrointestinal bleeding, hypertension, heart failure, congestive and renal insufficiency, which proves this treatment as unsafe.[7,12,13]

A class of drugs, SYSADOAs- Systemic slow acting drugs in OA, which contains glycosaminoglycans (GAGs) and their precursors, like chondroitin sulphate, glucosamine sulphate and hyaluronic acid are also used to treat OA. These drugs provide building blocks for regenerating cartilage without much adverse effects. But the action of these drugs depends upon their assimilation by body, which is responsible for their slower mode of action. Also, these agents do not act upon immune-mediated inflammatory cartilage destruction, thus showing less effectiveness.[11] Hence there is a need for safer and tolerable kinds of therapy which along with symptomatic relief, also minimize cartilage destruction and initiate regeneration of cartilage.

NATIVE COLLAGEN TYPE II AND AFLAPIN

Native (undenatured) collagen type II (NC II) is non-hydrolyzed collagen having intact triple helix structure with active epitopes and antigenicity. The extraction process of NC-II is a crucial mechanism using very little or no heat just to concentrate and make the collagen soluble. The native, triple helix of NC-II with intact antigenic sites (epitopes) is the biologically active form with potential for immunomodulation.[3,14,15] Various clinical and preclinical studies have been conducted to evaluate the effectiveness of NC-II in OA. These studies have demonstrated the efficacy of NC-II over other treatment options and showed that NC-II is a safer option to treat OA and also helps in regeneration of cartilage. NC-II acts through an oral tolerance mechanism which prevents cartilage damage by own immune system. It has also been shown that a small amount of NC-II can be sufficient to modulate joint health in OA.[9,15]

Boswellia serrata is a medicinal plant which commonly occur in different parts of India. Boswellia serrata gum resin was used traditionally as a remedy for multiple inflammatory diseases. The most pharmacologically active components of gum resin extracted from Boswellia serrata are pentacyclic triterpenoid molecules known as Boswellic acids. Amongst four major Boswellic acids, 3-O-acetyl-11-keto-betaboswellic acid (AKBA) is known as the most active component of Boswellia extract having anti-inflammatory, anti-arthritic, and anti-analgesic properties. It has been demonstrated that AKBA is a potent inhibitor of enzyme 5-LOX, which controls the biosynthesis of LTs from arachidonic acid.[9,13]

Aflapin is a novel synergistic composition of Boswellia gum resin and has higher composition of AKBA than other Boswellia extracts. Aflapin acts by reducing the levels of pro-inflammatory cytokines, decreasing cartilage degeneration, and improving cartilage regeneration via various mechanisms, while having a very good safety profile.[9]

MECHANISM OF ACTION

NC-II

The beneficial effect of NC-II on osteoarthritic cartilage can be mainly explained by the mechanism of oral tolerance. Oral tolerance can be elucidated as the immune process of the body to help distinguish between harmless compounds (e.g., dietary proteins, intestinal bacteria) and potentially harmful foreign invaders.[14] The mechanism is played out in the gut-associated lymphoid tissue (GALT) which is made up of mesenteric lymph nodes and patches of lymphoid tissue (Peyer's patches) in the duodenum. Oral intake of small amounts of three-dimensional, glycosylated NC-II with active epitopes leads to interaction with Peyer's patches and send required signals to induce immune tolerance.[16] Compounds from the gut lumen are screened by Peyer's patches and body's immune response is switched on or off depending on the compound. Interaction of intact active isotope of NC-II with Peyer's patches attenuates the immune response that is responsible for destroying joint cartilage.[3]

At a cellular level, oral intake of NC-II transforms naïve T-cells into T regulatory (Treg) cells in Peyer's patches. After migration via circulation, these Treg cells recognize type II collagen in joint cartilage and secrete anti-inflammatory cytokines such as TGF- β, IL-4 and IL-10. In this way, NC-II administration ultimately promotes endogenous type-II collagen to secrete anti-inflammatory cytokines, by transforming naive T-cells into Treg cells.[9] Transformation of naïve T-cells into Treg cells was demonstrated by an *in vitro* study conducted with lymphocyte cell culture of collagen-induced arthritis (CIA) rats. After the treatment with NC-II, cell culture showed increased levels of Treg cells, IL-4 and TGF- β whereas Th17 cells, IL-2 and IL-17 levels were observed to be decreased.[17] In an *in vitro* study, cartilage and synovial tissue of OA patients were co-cultured with polymerized-collagen for 7 days, which induced an increase of 3-6-fold cell proliferation and type II collagen expression. Treatment with collagen showed inhibition of pro-inflammatory cytokines (IL-1β, TNF-α) and upregulation of the levels of anti-inflammatory cytokine-IL-10.[18] Also, anti-inflammatory cytokine secretion by Treg cells has been evidenced in a study, in which collagen-induced arthritis (CIA) mice were treated with type II collagen-specific Treg cells. These Treg cells upregulated secretion of anti-inflammatory cytokines like IL-10, with inhibition of CD4+ effector T cells and concomitant reduction of IFN-γ levels.[19] It has been further demonstrated in a study with human chondrocytes, that the anti-inflammatory action of IL-10 protects against damage from tumor necrosis factor-alpha (TNF- α), a significant pro-inflammatory mediator in OA.[20]

Oral tolerance due to NC-II also acts via suppressing T helper cells and MMPs leading to collagen restoration and ECM synthesis.[14] In a macrophage and chondrocyte cell culture study, squid collagen (SC II) was found to help in

the production of type II collagen and GAG. SC II activates macrophages by inducing M2 polarization, which in turn promotes the expression of pro-chondrogenic genes (TGF-β and IGF) leading to the improvement in microenvironment around chondrocytes. Increased ratio of M2 macrophages, elevated levels of ant-inflammatory cytokines (TGF-β 1 and TGF-β 3) in synovial fluid and inhibition of chondrocyte apoptosis and MMP-13 production was observed in OA rats when treated with SC II.[21]

In another study, chicken collagen type II was administered orally to rats with induced OA. After treatment, reduction was observed in the levels of MMPs along with the expression of their respective mRNAs in articular cartilage. Morphological changes of osteoarthritic cartilage were also restricted leading to the inhibition of cartilage degradation.[22] Thus, through the mechanisms discussed above, NC-II can not only prevent the immune system from injuring its joint cartilage, but also promote cartilage repair and regeneration. Different mechanisms of NC-II and their evidence are summarized in Table 1.

Aflapin

The resinous part of Boswellia serrata consists of four major pentacyclic triterpenic acids, i.e., β-boswellic acid, acetyl-β-boswellic acid (A β BA), 11-keto-β-boswellic acid and AKBA. Aflapin is a synergistic composition derived from Boswellia serrata which is selectively enriched with AKBA and specific B Serrata-non-volatile oils to increase bioavailability.[9] Being a selective and potent 5-LOX inhibitor, Aflapin, reduces LT formation. It leads to the reduction of pro-inflammatory mediators like IL-1, TNF-α, ICAM and MMPs which further downregulates cartilage destruction and increases GAGs formation which ultimately maintains balance in favor of cartilage regeneration.[5,9,11.]

Few *in vitro* studies in which various rat cell cultures were treated with Boswellia extracts, have demonstrated inhibition of LT production by Aflapin via 5-LOX inhibition. All products of 5-LOX, both from endogenous and exogenous arachidonic acid, were reduced.[23-25]

In OA, Th1 cells are responsible for the production of pro-inflammatory cytokines IL-2 and IFN-γ which further leads to cartilage degradation. A couple of *in vitro* studies evaluated effects of Boswellia serrata extract in cell cultures of murine solenocytes and human PBMC. Boswellia extract inhibited Th1 cell expression and potentiated the formation of Th2 cells which produces anti-inflammatory cytokines IL-4 and IL-10. It also inhibited NO production via suppression of inducible NO synthase.[26,27]

Pro-inflammatory cytokine IL-1β induces chondrocyte apoptosis leading to cartilage resorption. Aflapin decreases production of IL-1 β which leads to inhibition of chondrocyte apoptosis and increases GAG production.[5,28] AKBA inhibits nuclear factor- kB (NF-kB) in human peripheral monocytes and downregulates TNF-α expression.[29] The anti-inflammatory effect of Boswellia extract was demonstrated in three different preclinical studies of induced OA in murine models treated with Boswellia extract. The study results exhibited reduction in pro-inflammatory cytokines IL-1β, IL-6, TNF-α, IFN-γ and PGE2. Increased levels of anti-inflammatory cytokine IL-10 was also observed.[30,31] The different mechanisms of Aflapin and their evidence are summarized in Table 2.

Table 1: Mechanism of action of NC-II.

Action	Mechanism	Evidence
Oral tolerance (anti-autoimmune)	Transformation of naïve T-cells into Treg cells leading to secretion of anti-inflammatory cytokines by endogenous type -II collagen.[9]	In an *in vitro* study with lymphocyte cell culture of CIA rats treated with NC-II ⇒ ↑ levels of Treg cells, IL-4 and TGF- β. ↓ levels of Th17 cells, IL-2, IL-17.[17] In cartilage and synovial tissue of OA patients co-cultured with polymerized-collagen⇒↓ levels of IL-1β, TNF- α, ↑ levels of IL-10.[18] In CIA mice treated with type II collagen-specific Treg cells⇒↑ secretion of IL-10, ↓levels of IFN-γ.[19]
Promotes cartilage regeneration	Activates macrophages by inducing M2 polarization. Promotes the expression of pro-chondrogenic genes (TGF- β and IGF. Improvement in microenvironment around chondrocytes. Produce matrix component leading to cartilage repair.[21]	In a macrophage and chondrocyte cell culture treated with squid collagen ⇒↑ ratio of M2 macrophages, ↑ levels of TGF-β 1 and TGF-β 3, ↓ chondrocyte apoptosis and MMP-13 production, ↑ matrix components like type II collagen and GAG.[21]
Reduces cartilage degradation	Suppression of MMPs leading to reduced articular cartilage degradation.	In OA rats treated with chicken collagen type II ⇒ ↓levels of MMPs and expression of their respective mRNA, ↓articular cartilage degradation.[22]

Treg=T-regulatory cell, CIA=Collagen induced arthritis, NC-II=Native collagen type II, IL=Interleukin, TGF=Transforming growth factor, Th17=T helper 17 cell, OA-Oateoarthritis, TNF=Tumor necrosis factor, IFN=Interferon, MMP=Matrix metalloproteinase, GAG=Glycosaminoglycan.

Table 2: Mechanism of action of Aflapin.

Action	Mechanism	Evidence
Reduction of LT-mediated inflammation	Inhibition of 5-LOX reduces LT formation leading to the reduction of pro-inflammatory mediators which ultimately downregulates cartilage destruction.[5,9,11]	In studies with various rat cell cultures treated with Boswellia extracts ⇒ ↓ levels of all products of the 5-LOX (IL-1, TNF-α, ICAM and MMPs).[23-25]
Reduction of cell-mediated inflammation	Inhibition of Th1 cell mediated inflammation by reduction of (Th1) pro-inflammatory cytokines and increase in levels of (Th2) anti-inflammatory cytokines.	In murine solenocytes and human PBMC cell culture treatment with Boswellia serrata extract ⇒↓ levels of pro-inflammatory cytokines IL-2, IFN-γ and NO. ↑ levels of anti-inflammatory cytokines IL-4 and IL-10.[26,27]
Inhibition of chondrocyte apoptosis	Inhibition of pro-inflammatory cytokine IL-1β, leading to the inhibition of chondrocyte apoptosis and increases GAG production.[28]	Human primary chondrocytes (induced by IL-1β) when treated with Aflapin⇒↑GAG content, ↓chondrocyte apoptosis.[5,28] In OA rats treated with Boswellia serrata extract ⇒↓levels of IL-1β, IL-6, TNF-α, IFN-γ and PGE-2. ↑ levels of IL-10.[30,31]

LT=Leukotriene, 5-LOX=5-Lipoxygenase, IL=Interleukin, TNF=Tumor necrosis factor, ICAM=ICAM interstitial cell adhesion molecules, MMP=Matrix metalloproteinase, Th=T helper cell, PBMC=Peripheral blood mononuclear cell, IFN=Interferon, NO=Nitric oxide, GAG=Glycosaminoglycan, OA=osteoarthritis, PGE-2=prostaglandin-E2.

CLINICAL EVIDENCE

The efficacy, tolerability, and safety of treatment with NC-II and Aflapin either independently or in combination have been evaluated in various human clinical studies. Most of the clinical trials were conducted on knee OA patients since it is a more common and severe type of arthritis. In these trials, commonly measured parameters are Western Ontario McMaster (WOMAC), Lequesne functional index (LFI), visual analogue scale score (VAS) and short form-36 (SF-36). WOMAC measures five items for pain (scored across 0-20), two for stiffness (scored across 0-8), and 17 for functional limitation (scored across 0-68), which cover everyday activities; higher the scores, worse is the pain, stiffness, and functional limitations. LFI has 10 questions divided into three sections regarding pain, maximum distance walked and activities of daily living, with score ranging from 0 (no pain, no disability) to 24 (maximum pain and disability). VAS is a psychometric response scale which consists of a line, often 10 cm long, with verbal anchors at either end; it is often found to have higher assay sensitivity, in scoring pain. SF-36 is a 36-item health survey questionnaire, which used as an objective measure of the quality of life and covers eight scales-physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health.

A clinical trial was performed in 100 moderate-to-severe knee OA patients aged between 45-60 years. All the patients received a daily dose of NC-II 40 mg capsule on an empty stomach for 120 days. Results showed a significant reduction in the overall WOMAC, LFI, and VAS score. The NC-II also led to significant changes in the WOMAC individual subscales of pain, stiffness, and physical function.[32] The clinical safety and effectiveness of NC-II was also evaluated in a multicentric, non-interventional, real world evidence clinical study. Around 291 patients were diagnosed with knee OA and were prescribed NC-II 40 mg capsule (containing 1.2 mg NC-II) per day. Enrolled patients were observed for safety parameters such as the incidence of suspected adverse drug reaction (AR), suspected serious adverse drug reaction (SAR), significantly abnormal clinical signs and symptoms, significantly abnormal laboratory parameters and the effectiveness of NC-II was evaluated by change in WOMAC score and VAS score from baseline. Improved pain, stiffness and enhanced functional mobility was observed with significant improvement in WOMAC score. The safety results also demonstrated the treatment to be safe in the management of OA.[33]

A randomized, placebo controlled, double blind clinical study was conducted to assess joint function by changes in degree of knee flexion and knee extension after exercise. In this study, adult, healthy subjects between 30-65 years age group who had no prior history of arthritic disease or joint pain at rest but experienced joint discomfort with physical activity were enrolled. Around 55 subjects were distributed in one placebo group and another test group receiving 40 mg NC-II daily for the period of 4 months. After the completion of 4 months, improvement in average knee extension and knee flexion of exercise for longer duration without any joint discomfort was observed in NC-II group as compared to placebo and to baseline.[34]

Acetaminophen is an analgesic and one of the conservative treatment options for OA patients. In two different clinical studies, efficacy and safety parameters were evaluated for UC and acetaminophen combination in comparison with only acetaminophen treatment. One of these studies was a randomized, single blind, controlled clinical trial, in which 39 patients aged between 45-70 years, diagnosed with primary OA and knee pain were enrolled. One group of patients was administered a daily dose of 1500 mg

acetaminophen (AC group) and the other group was treated with a combined dose of 1500 mg acetaminophen and 10 mg native collagen II (AC+CII group) daily. The treatment was given to both the groups for a period of three months. Pain, function, and quality of life were assessed by various scales like VAS, WOMAC and SF-36. Significantly better improvements were observed in AC+CII group compared to AC group. Hence it can be suggested that native type II collagen treatment combined with acetaminophen is superior to only acetaminophen for symptomatic treatment of patients with knee OA.[35] The other study was a randomized controlled clinical trial, with 60 patients of similar age having the diagnosis of primary knee OA. Half of the patients were treated with acetaminophen and the remaining patients received Acetaminophen plus NC-II treatment for the period of 3 months. The results suggest that NC-II collagen treatment combined with acetaminophen has more marked effect when compared to only acetaminophen with respect to symptomatic relief. This was evidenced by significantly better improvement in WOMAC and VAS score after 3-month treatment compared to baseline.[15]

Glucosamine hydrochloride (glucosamine HCl) plus chondroitin sulfate (GC) is also a treatment option for OA. Two different clinical trials were conducted to evaluate the effectiveness of NC-II treatment against GC. In one randomized clinical trial, 52 patients with OA of the knee between 40-75 years of age were enrolled to evaluate effectiveness of NC-II against glucosamine HCl plus chondroitin sulfate. Half of the study population was administered with NC-II and the other half with glucosamine HCl plus chondroitin sulfate for 3 months. Specifically, both treatments reduced symptoms. But NC-II treated subjects showed a more significant reduction in WOMAC, VAS and LFI score as compared to glucosamine HCl plus chondroitin sulfate.[12] Another placebo-controlled 3-arm study with similar population was conducted to compare NC-II and GC. In this study, a total of 191 patients were distributed in placebo group, NC-II (40 mg/day) group and glucosamine hydrochloride plus chondroitin sulfate group. UC supplementation showed significantly better changes in pain, stiffness, physical function, and improvement in WOMAC scale compared to placebo and glucosamine HCl plus chondroitin sulfate.[36]

Prevalence of OA is more frequent in female patients than men. There are a couple of studies conducted with female subjects. One small study with 5 female patients having the symptoms of joint pain was conducted to evaluate the tolerance and symptomatic relief achieved by UC treatment. A single oral daily dose of NC-II on an empty stomach for 42 consecutive days was given to all 5 patients. Results demonstrated that the dose was well tolerated and a 26% reduction in perceived pain was observed in 4 out of 5 patients.[16] The other randomized, controlled, clinical trial involved 105 female patients diagnosed with knee OA, distributed in two groups-treatment group treated with daily 40 mg NC-II capsule for 90 days and control group. Significant reduction in WOMAC and VAS score was observed in UC treated group as compared to control and baseline. UC treatment also improved quality of life of OA patients by reducing pain and stiffness.[37]

A couple of studies were conducted to evaluate the properties of Aflapin against placebo. In a randomized double-blind placebo controlled clinical trial, 60 patients suffering from mild to moderate unilateral or bilateral OA of the knee between 40-80 years of age were enrolled. Half of the patients were treated with Aflapin 100 mg/day and half received placebo for the duration of 30 days. These patients were assessed for pain, stiffness, and physical function by using WOMAC, LFI and VAS scores. Aflapin showed significant improvement in pain and functional ability after just 5 days of treatment.[38] In another placebo controlled randomized study, 70 OA patients were equally distributed to Aflapin (100 mg/day) treatment group and placebo-control group. WOMAC, VAS and LFI scores showed significant and early improvement of pain relief in Aflapin treated group after 30 days of treatment. Aflapin supplementation also reduced circulating inflammatory and cartilage biomarkers, including MMP-3, TNFα, and high-sensitive C-reactive protein (hsCRP).[39]

There was 1 study conducted to demonstrate effect of Boswellia serrata extract on 30 knee OA patients. Being a randomized placebo-controlled crossover study, half of patients received either Boswellia extract/placebo in first 8-week period of study and after washout received cross over treatment in second period. In both study periods, patients receiving Boswellia extract reported decreased knee pain, increased knee flexion and walking distance.[40]

Amongst different boswellia extracts, 5- Loxin is also an active component with potential anti-OA action. A group of researchers conducted two different studies; the first study compared effectiveness of two different concentrations of 5-Loxin in OA patients. The second study was conducted to compare effectiveness of 5-Loxin and Aflapin. In the first study 75 OA patients in the 40-80 years' age group were equally distributed in 5-Loxin (100 mg/day) group, 5-Loxin (250 mg/day) group and placebo group. Both the doses of 5-Loxin showed clinically and statistically significant improvement in pain scores and physical function. However, early, and more significant improvements were observed in 250 mg treatment group. Also, there was significant reduction observed in synovial fluid matrix metalloproteinase 3.[41] In second study 60 OA patients above 40 years of age were equally distributed in 5-Loxin (100 mg/day) group, Aflapin (100 mg/day) group and placebo group. Both Aflapin and 5-Loxin showed significant reduction in pain and improved physical function as compared to placebo and baseline. Results were better in Aflapin group compared to 5-Loxin.[13]

NC-II and Aflapin combination therapy can also be a good option to prevent cartilage degradation and immunomodulation in OA. A real-world study was conducted to evaluate efficacy and safety of NC-II and

Aflapin combination in 40 patients with knee OA in the 40-80 years' age group. All the patients were treated with a daily dose of Aflapin (100 mg) plus NC-II (40 mg) capsule for the period of 90 days. The treatment with this combination showed significant reduction in pain and stiffness with improved physical functions from baseline. This was evaluated using WOMAC and VAS scores. Also, no significant adverse effects were observed with this combination therapy.[42] The clinical evidence with the use of NC-II and Aflapin are summarized in Table 3.

Table 3: Clinical evidence for NC-II and Aflapin in OA.

S. no.	Clinical trial design	Study population and size	Study treatment	Results
Clinical trials of NC-II				
1	• Open label • Single arm	• Age 45-60 years • Subjects with knee OA, n=100	• A 40 mg capsule of collagen (NC-II) daily. • Duration-120 days	• Significant decrease in WOMAC score, LFI and VAS score.[32]
2	• Multicentric • Non-interventional • Real-life study	• Patients with knee OA. Prescribed with NC-II n=291	• A 40 mg capsule of collagen (NC-II) daily. • Duration-90 days	• Significant improvement in pain, stiffness, functional mobility and WOMAC score. • NC-II is safe and efficacious in patients with OA.[33]
3	• Randomized • Placebo controlled • Double blind	• Age 30-65 years • Healthy subjects who had no prior history of arthritic disease or joint pain at rest but experienced joint discomfort with physical activity, n=55	• Group 1-placebo (n=28) • Group 2-NC-II 40 mg capsule daily (n=27) • Duration-120 days	• Significant improvement in average knee extension in NC-II treatment group. • NC-II groups able to exercise longer before experiencing any initial joint discomfort.[34]
4	• Randomized • Controlled • Single blind	• Age 45-70 years • Patients with OA and knee pain • n=39	• Group 1-Acetaminophen 1500 mg/day (n=19) • Group 2-acetaminophen 1500 mg/day + Native collagen type II 10 mg/day (n=20) • Duration-90 days	• Significant improvements in joint pain (VAS walking), function (WOMAC) and quality of life (SF-36) in AC+NC-II group as compared to AC group.[35]
5	• Randomized • Controlled	• Age 45-70 years • Subjects with knee OA • n=60	• Group A: 1500 mg/day of acetaminophen (n=30) • Group B: 1500 mg/day of acetaminophen + 10 mg/day of NC-II (n=30) • Duration-90 days	• Subjects on AC+ NC-II showed a significant reduction in total VAS and WOMAC scores.[15]
6	• Randomized	• Age 40-75 years • Subjects with OA of knee • n=52	• Group 1: NC-II (n=26) • Group 2: Glucosamine HCl plus chondroitin sulfate (n=26, G+C) • Duration-90 days	• NC-II treated subjects showed a more significant reduction in WOMAC, VAS and LFI score as compared to glucosamine HCl plus chondroitin sulfate.[12]
7	• Multicentre • Randomized • Placebo controlled • Double blind	• Age 40-75 years • Patient with OA of knee, n=191	• Group 1: NC-II, (n=63) • Group 2: GC plus chondroitin sulfate, (n=66) • Group 3-Placebo, (n=62) • Duration-180 days	• NC-II group demonstrated a significant reduction in overall WOMAC score and all three WOMAC subscales: pain, stiffness, physical function compared to placebo and GC.[36]
Clinical trials of Aflapin				
1	•Randomized • Double blind • Placebo-controlled	• Age 40-80 years • Patients with OA of knee, n=60	• Group 1-Aflapin 100 mg/day (n=30) • Group 2-Placebo, (n=30) • Duration-30 days	• Aflapin group showed clinically and statistically significant improvements in pain scores and physical function scores.[38]
2	• Randomized • Double blind • Placebo-controlled	• Patients with OA, • n=70	• Group 1- Placebo (n=35) • Group 2 - Aflapin 100 mg/day (n=35) • Duration-30 days	• Significant reduction in VAS, LFI and WOMAC scores. • Reduction in circulating MMP-3, TNFα and hsCRP.[39]

Continued.

S. no.	Clinical trial design	Study population and size	Study treatment	Results
Clinical trials of Aflapin				
3	• Randomized • Placebo controlled • Double blind • Crossover study	• Patients with OA of the knee • n=30	• Group 1: Boswellia serrata extract (n=15) • Group 2: Placebo (n=15) • Duration-8 weeks • After washout period groups were crossed over to receive opposite intervention	• Boswellia serrata extract treated group reported decreased knee pain, increased knee flexion and walking distance.[40]
4	• Randomized • Placebo controlled • Double blind	• Age 40-80 years • Patients with mild to moderate OA • n=75	• Group 1 - 5-Loxin® 100 mg/day (n=25) • Group 2-5-Loxin® 250 mg/day (n=25) • Group 3-Placebo (n=25) • Duration-90 days	• Significant and early improvements in pain score and functional ability were recorded in the treatment group supplemented with 250 mg 5-Loxin®.[41]
5	• Randomized • Double blind • Placebo-controlled	• Age 40-80 years • Patients with mild to moderate OA of the knee • n=60	• Group 1 - Aflapin 100 mg/day (n=30) • Group 2-Placebo (n=30) • Duration-30 days	• Aflapin treatment showed significant and early improvements in pain scores and the physical function scores.[38]
6	• Randomized • Double blind • Placebo-controlled	• Age ≥40 years • Patients with medial tibio-femoral OA. • n=60	• Group 1-Placebo, (n=20) • Group 2-5-Loxin, 100 mg/day (n=20) • Group 3-Aflapin, 100 mg/day (n=20) • Duration-90 days	• Significant and early decrease in VAS, LFI and WOMAC scores. • Reduced levels of MMP-3, TNFα and hsCRP.[39]
Clinical trial of NC-II + Aflapin				
1	• RWE study • Single arm • Multicentric • Open label	• Age 40-80 years • Subjects with OA of the knee • n=40	• All patients received a capsule of Aflapin and NC-II daily • Duration-90 days	• Significant improvement was observed in WOMAC pain, stiffness, and physical functions score.[42]

NC-II=Native collagen type II, OA=Osteoarthritis, WOMAC=Western Ontario McMaster, LFI=Lequesne functional index, VAS=Visual Analogue Scale, SF-36=Short Form-36, AC=Acetaminophen, GC=Glucosamine hydrochloride plus chondroitin sulfate, MMP=Matrix metalloproteinase, TNF=Tumor necrosis factor, hsCRP=high-sensitive C-reactive protein.

CONCLUSION

OA is a degenerative and chronic inflammatory disease that affects the entire joint tissue. The interplay between mechanical and immune pathways has an important role in the pathogenesis of OA. Current pharmacological modalities have limited effect on the underlying pathology and mainly act via symptom relief, and their long-term use is fraught with safety concerns. NC-II and Aflapin through their complimentary mechanisms, that target both the mechanical and immune pathologies, can serve as an effective alternative to conventional drugs in the treatment of OA. A growing body of evidence supports their mechanism and clinical benefit in OA.

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