

## Review Article

# Systemic enzyme therapy with trypsin, bromelain and rutoside in the management of arthritis: an overview

Abhay P. Kulkarni<sup>1\*</sup>, Himanshu A. Bendrey<sup>2</sup>

<sup>1</sup>Department of Orthopaedics, Rajiv Gandhi Medical College, Kalwa, Thane, Mumbai, Maharashtra, India

<sup>2</sup>Bendrey Ortho Clinic, Chembur, Mumbai, Maharashtra, India

**Received:** 27 May 2021

**Revised:** 17 June 2021

**Accepted:** 18 June 2021

### \*Correspondence:

Dr. Abhay P. Kulkarni,

E-mail: [dr.abhay.k.29@gmail.com](mailto:dr.abhay.k.29@gmail.com)

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

Chronic arthritis, including osteoarthritis and rheumatoid arthritis, is a growing major public health problem leading to disability and reduced quality of life. Analgesic and anti-inflammatory drugs form the mainstay of treatment for chronic arthritis. The protracted use of the conventional medications for their management is fraught with shortcomings, including safety concerns. Proteolytic enzymes and antioxidant combinations have been used empirically, since ages, in many of these conditions. There is a growing body of evidence indicating the beneficial effects exerted by the individual ingredients and their combinations on the pathophysiology of arthritis. The analgesic, anti-inflammatory, anti-edematous, anti-thrombotic and anti-oxidant properties of these substances have been demonstrated in multiple in vitro and animal models. Furthermore, the therapeutic use of proteolytic enzyme-antioxidant combination is also supported by clinical trials in arthritis and related disorders. Such studies have mostly been carried out on preparations consisting of combinations of trypsin, bromelain and rutoside. The results of various studies (placebo-controlled and comparisons with nonsteroidal anti-inflammatory (NSAIDs) drugs) in patients with arthritis suggest that oral therapy with such enzyme-antioxidant combination produces improvement in all major clinical parameters like swelling, pain and joint stiffness and have comparable efficacy to NSAIDs. Some clinical studies also evaluated their effect on biochemical markers like cytokines, interferons and prostaglandins and reported remarkable improvements. The overall data also indicates that the tolerability of the enzyme-antioxidant combination is better than conventional therapies.

**Keywords:** Proteolytic enzymes, Cytokines, Anti-inflammatory, Osteoarthritis, Rheumatoid arthritis

## INTRODUCTION

Arthritis is a joint disorder featuring inflammation and the term is used to describe pain, swelling and stiffness in a joint or joints. It affects the joint movement and ultimately impacts the overall mobility of the patients. There are several different types (over 100 identified) ranging from those related to wear and tear (such as osteoarthritis or OA) to those associated with inflammation resulting from a misdirected immune system (such as rheumatoid arthritis or RA). The other types include gout, ankylosing

spondylitis, lupus arthritis, infectious arthritis, juvenile arthritis, psoriatic arthritis. OA and RA are the commonest.<sup>1,2</sup>

OA is a chronic progressive degenerative musculoskeletal disorder characterized by the deterioration of cartilage in joints and affects various soft and hard tissues around the joint.<sup>3</sup> The disease most commonly affects the joints in the knees, hands, feet and spine and is relatively common in shoulder and hip joints.<sup>4,5</sup> OA is the most common form of arthritis and is considered a major public health problem

and as per the global burden of disease study 2017, the condition affected 303 million people globally.<sup>6</sup> It was estimated to be the 10th leading cause of nonfatal burden affecting nearly 14% of adults aged 25 to 65 years and nearly 34% of adults over the age of 65 years.<sup>4</sup> Since knee is one of the most commonly affected joint, many of those with OA face impaired movement. The economic burden includes costs for adaptive aids and devices, medicines, surgery and time off at work. The prevalence of OA is increasing due to population ageing and an increase in related factors such as obesity. The prevalence in India is around 22-39%, with higher prevalence of symptomatic disease in women (18%) than men (9.6%) over 60 years of age.<sup>3</sup>

RA is chronic, progressive autoimmune disease characterized by persistent synovial and systemic inflammation associated with joint destruction, whose main symptom is chronic joint pain.<sup>7</sup> It initially affects small joints, progressing to larger joints and eventually the skin, eyes, heart, kidneys and lungs.<sup>8</sup> Incapacitation due to pain and articular rigidity are the major complaints of RA patients.<sup>9</sup> When RA is left uncontrolled, the RA patient may experience joint deterioration, severe disability, decreased quality of life, the onset of comorbidities and premature mortality.<sup>10</sup> Usually deformities and bone erosion caused due to damage to the joints is very painful for a patient.<sup>8</sup> The worldwide prevalence of RA in adults is approximately 0.5% to 1%. An estimated 20 million people had RA in 2017, with over a million new cases diagnosed each year, according to the global burden of disease study 2017. The estimated prevalence in India, based on data from four studies was 0.28% to 0.7%.<sup>10</sup>

## MANAGEMENT OF ARTHRITIS

The treatment for arthritis aims to control pain, minimize joint damage and improve or maintain function and quality of life. A range of medications and lifestyle strategies is implemented to achieve this and to protect joints from further damage. The modalities involve medications, non-pharmacologic therapies, physical or occupational therapy, splints or joint assistive aids, patient education and support, weight loss, surgery (including joint replacement). Pain-reducing medications are the mainstay for OA and most commonly include analgesics like acetaminophen, tramadol and narcotics containing oxycodone or hydrocodone and NSAIDs like ibuprofen and naproxen. Counterirritant creams and ointments containing menthol or capsaicin are also used due to property of modulating pain signals from the joint.<sup>11</sup> Most of these treatments are also applied to RA, along with anti-inflammatory medications such as corticosteroids and NSAIDs, disease-modifying anti-rheumatic drugs (DMARDs) and a relatively new class of drugs known as biologics. DMARDs slow or stop the immune system from attacking the joints. Examples include methotrexate and hydroxychloroquine. Used with DMARDs, biologic response modifiers are genetically engineered drugs that target various protein molecules involved in the immune

response. Examples include etanercept and infliximab. Corticosteroids like prednisone and cortisone reduce inflammation and act as non-specific suppressants of the immune system. In OA, the most frequently prescribed class of drugs is NSAIDs accounting for more than three-fourths of the prescriptions.<sup>12</sup>

## SAFETY CONCERNS

Since both the conditions are of chronic nature, the drugs are used over long periods of time. It has been recognized that chronic use of NSAIDs in older adults is fraught with risks. Older adults are at increased risk for adverse drug reactions (ADRs) due to age-related loss of physiological organ reserve, increased comorbidities, polypharmacy, and changes in pharmacokinetics. Studies of older adults show that chronic NSAIDs use increases the risk of peptic ulcer disease, acute renal failure and stroke/myocardial infarction. Moreover, chronic NSAIDs use can exacerbate a number of chronic diseases including heart failure and hypertension and can interact with a number of drugs.<sup>13</sup> The other frequently used class of analgesics is opioids, about which there is growing concern due to their narrow therapeutic margin, tolerance, dependence and adverse events such as delirium, somnolence and falls.<sup>14</sup> Methotrexate, which remains the anchor therapy in RA is frequently associated with neurotoxicity with cognitive impairment, gastrointestinal effects like mucositis, mouth ulceration, nausea and diarrhea. The less frequent but more serious effects include hepatotoxicity, neutropenia, lymphopenia, pulmonary abnormalities and higher risk of infection.<sup>15</sup>

Systemic enzyme therapy (SET) with proteolytic enzymes-flavonoid combinations have been used in traditional medicine for a long time. The therapeutic use of SET is empirically based but is also supported by scientific studies. Advances in the fields of immunology, biochemistry and molecular biology in the last few decades have led to better understanding of the mechanisms by which SET exerts the desired effects. Orally administered formulations of the combination of proteolytic enzymes (bromelain, trypsin) and the flavonoid rutoside (rutin) have been used since decades as natural anti-inflammatory agents. Few different formulations of this combination are dispersible tablets (Disperzyme®), enteric-coated tablets (Phlogam®) are approved for use in India. This review provides an overview of preclinical and clinical studies of SET comprising of the proteolytic enzymes trypsin and bromelain along with the flavonoid rutoside, in the management of arthritis and related conditions, especially OA and RA.

## PATHOPHYSIOLOGY

### *Osteoarthritis*

Current research has demonstrated that inflammation, with the presence of inflammatory cell infiltration is one of the key factors leading to the destruction of cartilage in OA.

The pathogenesis is therefore frequently linked to changes in chondrocyte activities including proliferation, matrix deposition, inflammatory cytokine production and response to signaling molecules.<sup>16,17</sup> Many cytokines have been found in OA joints, in correlation with the severity of inflammation and these play various roles in disrupting the balance of catabolic and anabolic activity in joint tissues. Interleukin (IL)-1 $\beta$ , IL-6 and tumor necrosis factor (TNF)- $\alpha$  cytokines play the most important roles in pathogenesis and disease severity of OA, while IL-15, IL-17, IL-18, IL-21 and chemokines and their receptors such as MCP-1/CCL2, IL-8/CXCL8 and GRO- $\alpha$ /CXCL1 have also been implicated.<sup>18</sup> During the development of OA, catabolic activity is triggered by pro-inflammatory cytokines, including IL-1 $\beta$ , IL-6, IL-17 and TNF- $\alpha$ . Elevated inducible nitric oxide synthase (iNOS) levels in OA chondrocytes result in an excess of nitric oxide (NO), which suppresses proteoglycan and collagen synthesis in chondrocytes and mediates the induction of matrix-degrading matrix metalloproteinases (MMPs) by accelerating the catabolic cascade induced by IL-1 $\beta$  or TNF- $\alpha$ . Nuclear factor  $\kappa$ B (NF- $\kappa$ B) is activated by inflammatory cytokines, elicits the secretion of many degradative enzymes including MMPs and suppresses ECM synthesis molecules such as Sox9, thereby down-regulating the ECM components type II collagen and aggrecan. NF- $\kappa$ B also acts in a positive feedback loop to augment the catabolic process by stimulating NF- $\kappa$ B-mediated inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, and the chemokine IL-8 and receptor activator of NF- $\kappa$ B (RANK) ligand (RANKL) leading to ECM breakdown and subsequent cartilage destruction.<sup>18</sup>

### **Rheumatoid arthritis**

In RA, there is characteristic synovial immunopathology which involves both resident and infiltrating cells. The synovial tissue becomes hyperplastic, edematous and characterized by an inflammatory cell infiltrate of T and B lymphocytes, plasma cells, macrophages, neutrophils, mast cells, natural killer cells and dendritic cells in the synovial sub-lining.<sup>19</sup> Inflammation in RA involves the combination of an antigen with an antibody and complement causing the local release of chemotactic factors that attract leukocytes. The leukocytes phagocytize this complex and also release lysosomal enzymes causing injury to cartilage and other tissues. Prostaglandins are also released during this process.<sup>20</sup> The inflammatory cascade involves cytokines and other pro-inflammatory molecules activating key specific signaling pathways such as hypoxia inducible factor (HIF), NF- $\kappa$ B and Janus kinase-signal transducer and activator of transcription (Jak-STAT).<sup>19</sup> It has been found that during the process of inflammation in RA, various pro-inflammatory cytokines such as tumor TNF- $\alpha$  and IL-1 are released and deemed as vital markers for the RA progression. The TNF- $\alpha$  induces stimulation and endurance of joint inflammation while IL-1 was reported to initiate the joint destruction.<sup>21</sup> The synovial tissue undergoes significant neovascularization, facilitating an influx of lymphocytes and monocytes that

transform a typically acellular loose areolar membrane into an invasive tumor-like pannus. NF- $\kappa$ B and iNOS are important mediators of inflammatory response in human and animal models of arthritis and its over expression leads to the extracellular matrix degradation and excessive cartilage and bone resorption, ultimately leading to the irreversible damage to joints.<sup>22</sup> It has been shown that RA bloodstream neutrophils and monocytes overproduce oxygen and nitrogen reactive species.<sup>23</sup> This is a result of the abnormal cellular metabolism and mitochondrial dysfunction which actively induce inflammation through the increased production of reactive oxygen species. Key pro-inflammatory cytokines, chemokines and growth factors and their signaling pathways including NF- $\kappa$ B, Janus kinase-signal transducer are highly activated when immune cells are exposed to hypoxia in the inflamed rheumatoid joint.<sup>19</sup>

### **PHARMACOLOGY OF TRYPsin-BROMELAIN-RUTOSIDE**

The enzyme trypsin is an animal derived serine protease, known to possess anti-inflammatory and immunomodulating properties. Orally administered trypsin is adsorbed and can be demonstrated in the blood stream, resulting in specific esterase activity changes.<sup>24</sup> Around 10% of an orally administered trypsin dose ends up in the blood.<sup>25</sup> Bromelain is a complex mixture of cysteine proteases extracted from the fruit or stem of the pineapple plant. A range of beneficial properties including anti-inflammatory, analgesic actions, anti-edematous and anti-thrombotic effects have been reported with bromelain.<sup>26,27</sup> It is absorbed from the human intestines, attains highest concentration in the blood in an hour and remains biologically active with a half-life of ~6-9 hours.<sup>28</sup> Both trypsin and bromelain bind to anti-proteinases like  $\alpha$ 2-antitrypsin and  $\alpha$ 2-macroglobulin in plasma and are protected from auto-digestion and from degradation by other serum proteases with retention of enzymatic activity.<sup>24,25</sup> In the bound form, they remain confined to the blood stream due to the large size, but at sites of inflammation, due to increased vascular permeability, they can enter the interstitial compartment.<sup>25</sup> Rutoside is a natural non-toxic bioflavonoid with anti-allergic, anti-inflammatory, anti-oxidant, anti-microbial, antiviral, anti-cancer and anti-diarrheal activities. These properties of rutoside have been extensively used in human nutrition and medicine.<sup>21,29</sup> Rutoside is useful in conditions involving free radicals in their pathogenesis as it is one of the most effective inhibitors of oxygen radical overproduction.<sup>23</sup>

Bromelain has been shown to proteolytically block the activation of extracellular regulated kinase-2 (ERK-2) in T cells, thereby inhibiting T cell signaling and cytokine production.<sup>30</sup> Bromelain treatment of anti-CD3 stimulated CD4+ T cells reduced CD25 expression in a dose and time dependent manner, mediated by its proteolytic action. CD25 is a therapeutic target in inflammation, autoimmunity and allergy.<sup>31</sup> It effectively decreases IL-8-

induced neutrophil migration to sites of acute inflammation both *in vitro* and *in vivo* possibly by proteolytic removal of the CD128 chemokine receptor.<sup>32</sup> It possesses potent inhibitory activity against phospholipase A2, a key enzyme in the initiation of inflammation, which subsequently induces the section of arachidonic acid and leads to the formation of inflammation mediators. It has shown to cause dose-dependent decrease of prostaglandin F2 and thromboxane B2 levels and plasma exudation, in rat model of kaolin-induced inflammation via depletion of the plasma kallikrein system.<sup>33</sup> When given orally to rats with carrageenan-induced inflammation, bromelain led to significant decrease of both prostaglandin E and substance P (a neuropeptide associated with inflammatory processes and pain) concentrations in the exudate.<sup>34</sup> Its anti-inflammatory, anti-oxidant and proteolytic effects were also demonstrated by evaluation of macroscopic and histopathologic scores in rat model of intra-abdominal adhesions.<sup>35</sup> When wistar rats with RA were administered oral bromelain loaded nanostructured lipid carriers, there was remarkable decrease in paw edema, joint stiffness, mechanical allodynia and tissue damage along with alleviation of oxidative stress.<sup>36</sup>

Trypsin also possesses anti-inflammatory and immunomodulating properties. When T lymphocytes or macrophages are cultured *in vitro* in the presence of trypsin, three cell surface molecules, CD4, CD44 and B7-1 were found to be sensitive to cleavage. These molecules are important regulators of the T-cell response. When T cells are isolated from enzyme-treated mice and tested freshly *ex vivo* for their antigen-specific recall response, their dose response curve is significantly right-shifted, that is, their activation threshold is increased. Bromelain also selectively cleaves CD44, possibly potentiating trypsin's effect.<sup>25</sup> When peripheral blood mononuclear cells were isolated and cultured to differentiate fibrocytes and macrophages in serum-free media, trypsin appeared to act as profibrotic signals through protease-activated receptor-1 (PAR1) and protease-activated receptor-2 (PAR2) receptors, altering macrophage surface marker expression and the macrophage secretion profile towards an M2a phenotype. M2a macrophages are involved in wound healing and fibrosis.<sup>37</sup> When given orally, in Wistar rats with induced-RA, trypsin was able to reduce nociception and edema. This was evaluated by the measurement of paw elevation time during 1 min periods of stimulated walk and articular diameter.<sup>9</sup>

Rutoside treatment in arthritic rats caused significant reduction in paw diameter with dose-dependent lowering of lowering of cytokines, tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$  and transcription factors NF- $\kappa$ Bp65. A significant upsurge in the level of superoxide dismutase, glutathione peroxidase and glutathione were observed together with decrease in the level of malondialdehyde. In addition, histopathological examination showed that the inflammatory cells infiltration, synovial hyperplasia, pannus formation and cartilage and bone erosion had considerably improved on administration of rutoside.<sup>21</sup> In

another such study, rutoside markedly inhibited joint swelling and significantly decreased the free radical load in collagen-induced arthritis rats. The pretreatment of rutoside replenished glutathione and superoxide dismutase levels significantly and suppressed the accumulation of lipid peroxidation, nitric oxide, probably by scavenging free radicals, thereby helping in maintaining the integrity of cellular membranes in the injured cartilage.<sup>38</sup> In a collagen-induced arthritis model of rats, treatment with rutoside led to significant down-regulation in the NF- $\kappa$ B and iNOS expression. The arthritic score as well as in the nitric oxide and peroxide levels were reduced in the treated groups.<sup>29</sup> These findings are in line with another study in experimental arthritis in rats, where rutoside was found to be extremely effective in reducing edema both in acute and chronic phases along with reduction in nodules and ankylosis.<sup>20</sup> In a study on septic arthritis due to *Candida albicans* (*C. albicans*), rutoside was found to reduce approximately 45% of the edema at the peak day of septic arthritis and inhibit growth of *C. albicans*, with no hemolysis, suggesting that rutoside has both anti-arthritic and anti-fungal effects. This was mediated by rutoside's ability to inhibit nitric oxide production from macrophages and T-cells proliferation.<sup>22</sup> Rutoside is also known to block pain and inflammation by activating cGMP/PKG/ATP-sensitive potassium channels pathway in neurons and inhibiting NF- $\kappa$ B and inducing Nrf2 activation in immune cells and modulates the production of mediators by neutrophils, mast cells and monocytes.<sup>39</sup> When added to activated human macrophages, rutoside inhibited inflammation-related gene expression and the release of nitric oxide, TNF- $\alpha$ , IL-1 and IL-6. In a rat model, RU inhibited clinical signs of chronic arthritis, correlating with decreased levels of inflammatory cytokines detected in rat sera and macrophage supernatants.<sup>40</sup> When blood samples from 43 RA patients were treated with rutoside, it was observed that spontaneous and stimulated oxygen radical production by RA neutrophils was strongly inhibited.<sup>23</sup> The anti-inflammatory activity of rutoside was also evaluated by performing *in silico* molecular docking analysis with TNF- $\alpha$ . Rutoside was found to form a 6 hydrogen bond interaction with TNF- $\alpha$  providing the possible molecular mechanism of inhibition of TNF- $\alpha$  by rutoside.<sup>41</sup>

In a collagen-induced arthritis model in mice, the SET combination of trypsin, bromelain and rutoside was compared with ibuprofen, when administered orally twice daily. After 2 weeks, joints were scored for clinical, radiographic and histologic changes. Similar degree of amelioration of joint inflammation and accentuation of a prototypical Th2 cytokine (interleukin 5) was observed. However, SET, in addition, showed protective effect on articular cartilage, normalized the sialylation of IgG and anti-collagen antibody and fully restored Th1 (interferon- $\gamma$ ) synthesis unlike ibuprofen.<sup>42</sup> This same enzyme combination was studied in collagen-induced arthritis model in rats, alone and in combination with cyclosporin A. The combination therapy was shown to significantly inhibit both inflammation and destructive arthritis-

associated changes. Reduction of the radiographic scores was also more significant in the combination therapy group.<sup>43</sup>

## CLINICAL STUDIES WITH SET

The enzymes trypsin (with chymotrypsin), bromelain and rutoside have been studied, individually and in combination (as SET), in multiple clinical trials of rheumatic and related disorders.

Cohen and Goldman in 1964, investigated bromelain by a series of case reports on 28 patients, with moderate or severe rheumatoid or OA. It was observed that bromelain had positive clinical effects in 18 patients when consumed orally by RA patients in dosages of 20 or 40 mg for 3-4 times daily up to 13 months without any adverse events occurred.<sup>44</sup> In a double-blind, randomized study in 19 patients, with confirmed RA, SET over 12 months with pancreatin/papain/bromelain/trypsin/chymotrypsin led to significant decrease in circulating immune complexes. These findings were further corroborated in another study with 42 RA patients.<sup>45</sup>

The effect of orally administered proteinases on the level of cytokines was evaluated in a clinical study of 156 patients with RA. The study reported that the serum levels of interferons were reduced to almost normal values after 6 months of therapy in the enzyme-treated group (a combination of pancreatin/papain/bromelain/trypsin/chymotrypsin+methotrexate) compared with 2-3 times higher values in the control group (NSAID+methotrexate therapy). In addition, serum levels of IL-1 $\beta$  and TNF- $\alpha$ , were also reduced significantly more in the enzyme-treated group than in the control group.<sup>45</sup>

In a randomized, double-blind clinical study, SET was compared to diclofenac in 80 patients with knee OA, after 4 weeks of treatment. The endpoints were pain at rest, on motion, on walking, at night and pain tenderness. There were comparable improvements in these parameters with the two therapies. In both groups the common adverse events (AEs) reported were gastrointestinal complaints, and all the events subsided after stopping the drug.<sup>46</sup> Another randomized, single-blind study compared SET with diclofenac in 50 patients with knee OA over 3 week treatment. At the end of 3 week and at 4 week post-treatment, there was similar reduction in pain and swelling in both groups, while more patients in SET group experienced reduction in joint tenderness than with diclofenac.<sup>47</sup>

A pooled re-analysis of data from 6 trials in knee OA published from 2011 to 2015, that enrolled 774 patients, was published in 2016. All the trials included were prospective, randomized, double-blind, parallel-group studies in adult patients with moderate-to-severe OA of the knee treated for at least 3 weeks with SET or diclofenac. This analysis included 6 trials published from 2011 to 2015, that enrolled 774 patients. All patients had OA of the

knee confirmed by conventional radiography and/or tomography and in all 6 studies, primary efficacy analyses were based on the self-assessment of pain and functionality of the affected knee joint using the Lequesne algofunctional index (LAFI). This index is an internationally used validated patient questionnaire that includes three categories: pain and discomfort, maximum distance walked and activities of daily living and is recommended by the US food and drug administration and the European medicines agency. Secondary efficacy analyses addressed self-assessments of knee pain intensity at rest (PIR) and pain intensity in motion (PIM). The results demonstrated comparable efficacy and clinically relevant improvement with respect to knee-joint pain and pain-related restrictions in daily life functioning. Both treatment groups showed an improvement in more than 75% of patients. The PIR and PIM improved significantly in both groups and two-thirds of patients in both groups reported a relief PIM greater than the minimal clinically important difference (MCID) on a 11-point numeric rating scale, indicating clinically relevant treatment effects. SET had a better safety profile, characterized by a lower occurrence of AEs and AE-related treatment discontinuations. No SET related changes were identified from the laboratory data, while the diclofenac treatment was associated with changes in key hepatic enzymes in ~72.6% and red blood parameters in ~86.3% of patients.<sup>48</sup>

Around the same time, there was another randomized, single-blind 40 patient study assessing a 16 week treatment with bromelain in mild-to-moderate knee OA patients. The patients were randomized to receive oral bromelain (500 mg/day) or diclofenac (100 mg/ day). Improvement was observed in both groups as identified by Western Ontario and McMaster universities osteoarthritis index (WOMAC) index, pain subscales, stiffness subscales and function subscales and physical component of short-form 36 (SF-36). Reduction in lipid peroxidation and LPS-induced PGE2 production was observed at week 16 in bromelain group. There were two patients who dropped out of trial-both in the diclofenac group, due to intolerable dyspnea and heartburn. Bromelain was well tolerated. Its adverse effects were mild nausea, constipation, flatulence, diarrhea, dry mouth, headache and tiredness.<sup>5</sup>

There is also a study in which SET was combined with zinc supplementation and compared to diclofenac in 50 patients of knee OA after 6 weeks of treatment. Efficacy determined by WOMAC scores indicated that SET+zinc was equally effective as diclofenac in alleviating pain and reduction of edema.<sup>49</sup> More recently, there have been studies combining SET with diclofenac. In one study, 30 patients with symptomatic temporo-mandibular joint (TMJ) OA were randomly divided into three groups, 10 in diclofenac sodium, 10 in SET and 10 in the combination group. The results indicated that while both diclofenac and SET groups showed similar effectiveness in the management of pain, the combination group performed much better than both.<sup>3</sup>

The effect of daily bromelain supplementation on acute mild knee pain in otherwise healthy adults was also evaluated. This was done in an open, parallel, dose ranging, study conducted by means of postal questionnaires. One hundred and twenty-six adults aged 25 to 50 years were included in the study conducted in United Kingdom (UK), who had suffered knee pain on a regular basis for no longer than three months. Seventy seven volunteers completed the study amongst them, 43 had taken the lower and 34 the higher dose of bromelain. As a result, there is 59% decrease in the WOMAC final battery (a sum of pain, stiffness and physical function scores) was observed in the higher dose group after one month of intervention. There was a significant effect of bromelain on reducing symptoms of knee pain and improving well-being in study subjects.<sup>27</sup>

There is one study of SET in peri-arthritis humeroscapularis tendopathica. In this randomized, double-blind trial, SET and diclofenac were administered to 20 patients each for 3 weeks. The sum score of the various kinds of pain and dysfunction after 3 weeks decreased from 10.1 to 0.7 in the SET group and from 9.7 to 1.6 in the diclofenac-treated group.<sup>45</sup> An oral enzyme combination was also compared to diclofenac in a study of 50 patients with closed fracture lower end radius. The combination of bacterial proteases, papain, bromelain, vitamin C and rutoside showed better reduction of edema, although pain relief was better with diclofenac. The combination was reported to be safer than diclofenac.<sup>50</sup>

## CONCLUSION

Due to the chronic nature of rheumatic conditions and protracted nature of treatment, safer alternatives to conventional drugs are highly desirable. SET with proteolytic enzymes-flavonoid combinations have been used empirically for such rheumatic conditions for decades. It works through a combination of anti-inflammatory, anti-oxidant, anti-edematous and analgesic effects. A large and increasing body of evidence regarding their mechanisms and clinical benefit is available in literature. It has been shown that behind the empirically supported clinical results are a complex set of regulatory processes, which previously were unknown. Specifically, the effect of proteolytic enzymes on the cytokine network and their action at the level of the cell membrane both in terms of cellular adhesion as well as modulation of cellular receptors has been described. SET has been shown to be much better tolerated when compared to NSAIDs and do not have the same gastrointestinal effects, and appear to be a safer alternative, especially for older patients, requiring long-term treatment.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: Not required*

## REFERENCES

- Mitra S. Arthritis: classifications, nature and cause-a review. *Am J Biopharmacol Biochem Life Sci.* 2013;2(3):1-25.
- Senthelal S, Li J, Goyal A, Bansal P, Thomas M. Arthritis. Treasure Island (FL): StatPearls Publishing; 2020.
- Jayachandran S, Khobre P. Efficacy of bromelain along with trypsin, rutoside trihydrate enzymes and diclofenac sodium combination therapy for the treatment of TMJ osteoarthritis-a randomised clinical trial. *J Clin Diagn Res.* 2017;11(6):9.
- Bolten W, Glade M, Raum S, Ritz B. The safety and efficacy of an enzyme combination in managing knee osteoarthritis pain in adults: a randomized, double-blind, placebo-controlled trial. *Arthritis.* 2015;2015:1-7.
- Kasemsuk T, Saengpetch N, Sibmooh N, Unchern S. Improved WOMAC score following 16-week treatment with bromelain for knee osteoarthritis. *Clin Rheumatol.* 2016;35(10):2531-40.
- Kloppenborg M, Berenbaum F. Osteoarthritis year in review 2019: epidemiology and therapy. *Osteoarthritis Cartilage.* 2020;28(3):242-8.
- Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *E Clin Med.* 2020;29:100587.
- Bullock J, Rizvi S, Saleh A, Ahmed S, Do D, Ansari R, et al. Rheumatoid arthritis: a brief overview of the treatment. *Med Princip Pract.* 2018;27(6):501-7.
- Lucena F, Foletto V, Mascarin L, Tonussi C. Analgesic and anti-edematogenic effects of oral trypsin were abolished after subdiaphragmatic vagotomy and spinal monoaminergic inhibition in rats. *Life Sci.* 2016;166:60-5.
- Handa R, Rao U, Lewis J, Rambhad G, Shiff S, Ghia C. Literature review of rheumatoid arthritis in India. *Int J Rheum Dis.* 2016;19(5):440-51.
- Wu Y, Goh E, Wang D, Ma S. Novel treatments for osteoarthritis: an update. *Open Access Rheumatology: Research and Reviews.* 2018;10:135-40.
- Gupta R, Malhotra A, Malhotra P. Study of prescription pattern of drugs used in the treatment of osteoarthritis in a tertiary care teaching hospital: an observational study. *Int J Res Med Sci.* 2018;6(3):985.
- Marcum Z, Hanlon J. Recognizing the risks of chronic nonsteroidal anti-inflammatory drug use in older adults. *Ann Longterm Care.* 2010;18(9):24-7.
- Machado-Duque M, Ramírez-Valencia D, Murillo-Muñoz M, Machado-Alba J. Trends in opioid use in a cohort of patients with rheumatoid arthritis. *Pain Res Manag.* 2020;2020:3891436.
- Bird P, Griffiths H, Littlejohn G. Methotrexate in rheumatoid arthritis: efficacy and safety. *J Pharmacovigil.* 2014;02(2):1-4.

16. He Y, Li Z, Alexander P, Ocasio-Nieves B, Yocum L, Lin H, et al. Pathogenesis of osteoarthritis: risk factors, regulatory pathways in chondrocytes, and experimental models. *Biology.* 2020;9(8):194.
17. Johns Hopkins Arthritis Center. Fact sheet: Osteoarthritis: Pathophysiology. Available at: <https://www.hopkinsarthritis.org/arthritis-info/osteoarthritis/oa-pathophysiology/>. Accessed on 14 May 2021.
18. Kim J, Yoo J, Kim H. Therapeutics in osteoarthritis based on an understanding of its molecular pathogenesis. *Int J Mol Sci.* 2018;19(3):674.
19. Veale D, Orr C, Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. *Semin Immunopathol.* 2017;39(4):343-54.
20. Guardia T, Rotelli A, Juarez A, Pelzer L. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Farmaco.* 2001;56(9):683-7.
21. Sun C, Wei J, Bi L. Rutin attenuates oxidative stress and proinflammatory cytokine level in adjuvant induced rheumatoid arthritis via inhibition of NF- $\kappa$ B. *Pharmacology.* 2017;100(1-2):40-9.
22. Prasad R, Prasad S. A review on the chemistry and biological properties of Rutin, a promising nutraceutical agent. *Asian J Pharm Pharmacol.* 2019;5(1):1-20.
23. Ostrakhovitch E, Afanas'ev I. Oxidative stress in rheumatoid arthritis leukocytes: suppression by rutin and other antioxidants and chelators. *Biochem Pharmacol.* 2001;62(6):743-6.
24. Lorkowski G. Gastrointestinal absorption and biological activities of serine and cysteine proteases of animal and plant origin: review on absorption of serine and cysteine proteases. *Int J Physiol Pathophysiol Pharmacol.* 2012;4(1):10-27.
25. Lehmann P. Immunomodulation by proteolytic enzymes. *Nephrol Dial Transplant.* 1996;11(6):952-5.
26. Brien S, Lewith G, Walker A, Middleton R, Prescott P, Bundy R. Bromelain as an adjunctive treatment for moderate-to-severe osteoarthritis of the knee: a randomized placebo-controlled pilot study. *QJM.* 2006;99(12):841-50.
27. Walker A, Bundy R, Hicks S, Middleton R. Bromelain reduces mild acute knee pain and improves well-being in a dose-dependent fashion in an open study of otherwise healthy adults. *Phytomed.* 2002;9(8):681-6.
28. Rathnavelu V, Alitheen N, Sohila S, Kanagesan S, Ramesh R. Potential role of bromelain in clinical and therapeutic applications. *Biomedical Rep.* 2016;5(3):283-8.
29. Gul A, Kunwar B, Mazhar M, Faizi S, Ahmed D, Shah M, et al. Rutin and rutin-conjugated gold nanoparticles ameliorate collagen-induced arthritis in rats through inhibition of NF- $\kappa$ B and iNOS activation. *Int Immunopharmacol.* 2018;59:310-7.
30. Mynott T, Ladhams A, Scarmato P, Engwerda C. Bromelain, from pineapple stems, proteolytically blocks activation of extracellular regulated kinase-2 in T cells. *J Immunol.* 1999;163(5):2568-75.
31. Secor E, Singh A, Guernsey L, McNamara J, Zhan L, Maulik N, et al. Bromelain treatment reduces CD25 expression on activated CD4+ T cells in vitro. *Int Immunopharmacol.* 2009;9(3):340-6.
32. Fitzhugh D, Shan S, Dewhirst M, Hale L. Bromelain treatment decreases neutrophil migration to sites of inflammation. *Clin Immunol.* 2008;128(1):66-74.
33. Kumakura S, Yamashita M, Tsurufuji S. Effect of bromelain on kaolin-induced inflammation in rats. *Eur J Pharmacol.* 1988;150(3):295-301.
34. Gaspani L, Limioli E, Ferrario P, Bianchi M. In vivo and in vitro effects of bromelain on PGE2 and SP concentrations in the inflammatory exudate in rats. *Pharmacology.* 2002;65(2):83-6.
35. Sahbaz A, Aynioglu O, Isik H, Ozmen U, Cengil O, Gun B, et al. Bromelain: a natural proteolytic for intra-abdominal adhesion prevention. *Int J Surg.* 2015;14:7-11.
36. Sharma M, Chaudhary D. Exploration of bromelain laden nanostructured lipid carriers: an oral platform for bromelain delivery in rheumatoid arthritis management. *Int J Pharm.* 2021;594:120176.
37. White M, Gomer R. Trypsin, Trypsinase, and Thrombin Polarize Macrophages towards a Pro-Fibrotic M2a Phenotype. *PLoS One.* 2015;10(9):0138748.
38. Umar S, Mishra N, Pal K, Sajad M, Ansari M, Ahmad S, et al. Protective effect of rutin in attenuation of collagen-induced arthritis in Wistar rat by inhibiting inflammation and oxidative stress. *Indian J Rheumatol.* 2012;7(4):191-8.
39. Ferraz C, Carvalho T, Manchope M, Artero N, Rasquel-Oliveira F, Fattori V, et al. Therapeutic potential of flavonoids in pain and inflammation: mechanisms of action, pre-clinical and clinical data, and pharmaceutical development. *Molecules.* 2020;25(3):762.
40. Kauss T, Moynet D, Rambert J, Al-Kharrat A, Brajot S, Thiolat D, et al. Rutoside decreases human macrophage-derived inflammatory mediators and improves clinical signs in adjuvant-induced arthritis. *Arthritis Res Ther.* 2008;10(1):19.
41. Sujitha B, Kripa K. Anti-arthritis and anti-inflammatory polyphenols from *Caryota urens* L.: a molecular docking analysis. *Res J Pharm Technol.* 2020;13(9):4269-73.
42. Chintalacheruvu S, Urankar-Nagy N, Petersilge C, Abdul-Karim F, Emancipator S. Treatment of collagen induced arthritis by proteolytic enzymes: immunomodulatory and disease modifying effects. *J Rheumatol.* 2001;28(9):2049-59.
43. Rovenska E, Svik K, Stancikova M, Rovensky J. Inhibitory effect of enzyme therapy and combination therapy with cyclosporin A on collagen-induced arthritis. *Clin Experiment Rheumatol.* 2001;19(3):303-9.
44. Brien S, Lewith G, Walker A, Hicks S, Middleton D. Bromelain as a treatment for osteoarthritis: a review

- of clinical studies. *Evid Based Complement Alt Med*. 2004;1(3):251-7.
45. Leipner J, Iten F, Saller R. Therapy with proteolytic enzymes in rheumatic disorders. *Bio Drugs*. 2001;15(12):779-89.
  46. Singer F, Oberleitner H. Drug therapy of activated arthrosis. On the effectiveness of an enzyme mixture versus diclofenac. *Wiener Medizinische Wochenschrift*. 1996;146(3):55-8.
  47. Tilwe G, Beria S, Turakhia N, Daftary G, Schiess W. Efficacy and tolerability of oral enzyme therapy as compared to diclofenac in active osteoarthritis of knee joint: an open randomized controlled clinical trial. *JAPI*. 2001;49:617-21.
  48. Ueberall M, Mueller-Schwefe G, Wigand R, Essner U. Efficacy, tolerability, and safety of an oral enzyme combination vs diclofenac in osteoarthritis of the knee: results of an individual patient-level pooled reanalysis of data from six randomized controlled trials. *J Pain Res*. 2016;9:941-61.
  49. Bai KS, Naik PS, Naik MAB, Riyaz N, Kushbu D. An observational study comparing the efficacy and tolerability of trypsin, bromelain, rutoside and zinc combination with diclofenac sodium in patients suffering from osteoarthritis knee attending orthopedic OPD, GGH, Anantapuramu. *Int J Curr Adv Res*. 2015;4(12):545-8.
  50. Acharya T, Trivedi M, Mehta D, Chhaiya S, Gandhi S. A comparative study of effect on reducing pain, inflammation and side effect of combination of enzymes (bacterial proteases, papain, bromelain, vitamin C and rutin) versus conventional non-steroidal anti-inflammatory drugs (diclofenac) in patients of closed fracture lower end radius coming at orthopaedic department of a tertiary care hospital. *Int J Basic Clin Pharmacol*. 2016;5(3):1017.

**Cite this article as:** Kulkarni AP, Bendrey HA. Systemic enzyme therapy with trypsin, bromelain and rutoside in the management of arthritis: an overview. *Int J Res Orthop* 2021;7:1062-9.