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Review Article

RHEUMATOID ARTHRITIS: MOLECULAR BASIS AND CURES FROM NATURE

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ABSTRACT

Incidences of arthritic diseases in human have seen recent increases which are thought to have resulted from a complex interplay of several factors, such as changes in lifestyle, nutritional insufficiencies, aging and genetic factors. These putative factors possibly lead to different arthritic diseases in humans affecting 2-5% of the total population in India. This group of diseases results in serious malfunction and structural abnormalities in the patient body leading to permanent and substantial immovability of joints. Conventional medicinal systems usually elicit various side effects in which the defence mechanism of the body i.e. the immune system is compromised. In the last few decades many alternative medicinal systems have been developed that show promising effects on treating such diseases. Many purified compounds from natural origin, both from plants and animal sources have shown promise and many new compounds are continuingly being identified which have no marked side effects. In the light of modern science and technology, different natural products and ethnic practices that ensure health, seem to be the best weapon to combat these diseases. Endemic as well as naturalized plants from India have been screened by several groups for their anti-arthritic activities. The review summarizes our current knowledge on the molecular basis of Rheumatoid Arthritis and discusses the efficacious roles of those natural products, especially of plant origin, in arthritic conditions.

Keywords: Rheumatoid arthritis, Medicinal plant.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder affecting principally the flexible (synovial) joints and sometimes adjacent tissues and organs. In severe condition of the disease, if kept untreated, it may result in a disabling and painful condition, which can lead to substantial loss of function and mobility. This debilitating disease is prevalent in all over the world including in Indian population. Considering common symptoms, there is no strong difference between symptoms of RA and other joint arthritic diseases like gout, osteoarthritis or ankylosing spondylytis, so there lays a great difficulty in treatment. Early theories on the RA pathogenesis focused on autoantibodies and immune complexes [1]. But recent reviews focus on autoantibodies as well as T-cell mediated antibody responses and T-cell independent cytokine networks [1]. Cytokines are now regarded as the very important regulating factors in rheumatoid arthritis. Studies from cytokine network interruption by anti-TNF-α antibody have shown positive results in collagen-induced arthritic rat models [2]. Cytokines are also responsible for the bone destruction near the synovial joint where Cytokines like IL-1, IL-17 have been shown to be responsible for matrix destruction [3]. Till now, no specific drug is available for treating RA. Presently few immunosuppressive drugs like glucocorticoids are found to be effective to suppress the disease expression and inflammation of the synovial joint, which are disadvantageous due to their side effects [4].

Ayurveda or ayurvedic medicinal system, a system of traditional medicine native to India, is a form of complementary alternative medicine that generally depends on different plants and plant-derived products. However, the science underlying the working principle of such herbal products are not revealed in most cases and they face a problem of improper clinical trials. Many common Indian plants are being studied in different laboratories for their anti-arthritic and anti-inflammatory properties, which include Green tea (*Camellia sinensis*), Ashoka (*Saraca asoca*), Tulsi (*Ocimum sanctum*), Devdaru (*Cedrus deodara*) and many others. Few plants have been found to be effective in reducing inflammation and arthritic swelling. Several other plants or plant products are also being explored for their efficacy. Cytokine gene regulation, cellular signalling mechanism and mechanism of bone destruction are being extensively studied for that purpose.

Classification criteria are needed in population studies to establish the epidemiology of the disease, to define entry criteria for clinical trials, to evaluate whether individuals have the specific disease, and to train medical students. The methodology of development of classification criteria has gradually been refined over the last 50 years or so. According to American College of Rheumatology (ACR) criteria (1987) a total of 41 rheumatologists from university and private practice were asked to provide details of patients aged 16 years and older whom they considered to have RA and of the next consecutive patients who did not have RA or a localized rheumatic condition [5]. Based on those details, the following table (table 1) presents the classification criteria for arthritic diseases:

Table 1: Classification criteria for arthritic diseases according to the American College of Rheumatology (ACR) criteria, proposed in 1987 [5]

Types		Description
1.	Morning stiffness	Morning stiffness in and around the joints, lasting at least for 1 hour before maximal improvement.
2.	Arthritis in three or more joint areas	Soft tissue swelling or fluid (not bony overgrowth) observed by a physician, present simultaneously for at least 6 weeks.
3.	Arthritis of hand joints	Swelling of wrist MCP or PIP for at least 6 weeks.
4.	Symmetrical arthritis	Simultaneous involvement of the same joint areas (defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry for at least 6 weeks.
5.	Rheumatoid nodules	Subcutaneous nodules over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician.
6.	Rheumatoid factor	Detected by a method positive in less than 5% normal controls.
7.	Radiographic changes	Typical of RA on postero-anterior hand and wrist radiographs which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

Rheumatoid arthritis is defined by the presence of four out of the seven criteria (table 1) that were developed by the American College of Rheumatology (ACR) in 1987 [5]. Symptoms can vary greatly depending on the type of arthritis and the individual concerned. In the most common forms, joints of the knees, fingers, wrists, ankles, hips, and/or elbows become stiff, swollen, tender, and painful. This pain can be greater in the morning, or get worse as the day goes on. Often fatigue occurs and is sometimes accompanied by a sleep disorder. Painless lumps under the skin, called rheumatoid nodules, can be felt in RA. In juvenile rheumatoid arthritis, fever, rash, and anaemia occur. The symptoms can also be cyclical that appear, disappear and reappear with time, where reappearance is often termed "flare up" phase. This chronic pain ranges from mild to severe and can last for the lifetime.

As it is an autoimmune disease, genetic factors are predisposed in person to initiate the disease. For instance, in some people with RA, some specific alleles of HLA genes that affect the immune system are found in high frequency. Along with that, lifestyle also plays an important role in developing RA. The onset of symptoms can occur at a wide range of ages spanning from youth to the elderly although most forms become more common with ages. Older people can get arthritis because of general wear and tear on joints, as well as a direct effect of previous injuries like injuries related to sports. Likewise, obesity can also increase stress to joints and can, therefore, contribute to arthritis. RA is more prevalent in women, while other types of arthritic diseases, like gout, occur more frequently in men, therefore there seems to exist a role of hormones of the affected individual in eliciting the disease.

Epidemiology of rheumatoid arthritis

The rheumatoid arthritic disease affects 0.5–1% of the population in the industrialized world and commonly leads to significant disability and quality of life is consequently reduced [6]. It is 2 to 3 times more frequent in women than in men and can start at any age, with a peak incidence between the fourth and sixth decade of life. Its prevalence in India ranges from 0.28% in the urban population to 0.55% in the rural population as per the survey done [7] but the actual incidence range may vary from 2-5% in the country.

Pathogenesis of rheumatoid arthritis

RA is regarded as an autoimmune disease. The disease has some strong association with several types of auto-antibodies like rheumatoid factor (RF), anti-perinuclear factor (APF) and anti-keratin antibodies (AKA), anti-collagen antibodies, antibodies to nuclear antigens such as Epstein-Barr nuclear antigen and RA33, anti-Sa, and anti-p68 antibodies [4]. Most of these antibodies react/interact with citrullinated proteins [8]. But it is still unknown whether such autoantigens initiate the T-cell activation cascade from the very beginning by any signalling pathway to form inflammatory changes, or contribute to the disease at a later stage to boost and/or perpetuate the disease.

RA has a polygenic basis and 31 risk loci have been identified. The presence of some of those loci in different combinations increases the chance of the disease by several folds [9]. In such genetically predisposed individuals the innate immune response is possibly activated by events such as the triggering of dendritic cells (DCs) through TLRs (several of which are known to be expressed on synovial cells) by exogenous material or by a combination of foreign stimuli together with autologous antigens [1, 10, 11]. Synovial dendritic cells activated by TLR ligands can migrate to lymph nodes where primed T-cells can be biased towards the T_H1 phenotype. The synovial membrane is infiltrated by T-cells, which produce IL-2 and IFN-γ. So the T-cell response attains a T_H1 bias. Further, T_H17 cells may be an important effect or T-cell subset in RA [12]. These T-cells activate monocytes, macrophages and synovial fibroblasts through cell–cell contact and activation by different cytokines, such as IFN-γ, TNF-α and IL-17 [13, 14]. These immune cells then overproduce pro-inflammatory cytokines — mainly TNF-α, IL-1 and IL-6 — which seem to constitute the pivotal event leading to chronic inflammation.

Many other cytokines and chemokines are involved in RA progression, including IL-15, IL-18 and angiogenic factors. These molecules, after binding to their specific receptors, can regulate various signal transduction cascades, such as the MAPK, nuclear factor-κB (NF-κB) or Jak/STAT pathways, which ultimately lead to the activation/inhibition of transcription factors or subsequent induction of genes responsible for mediation of inflammation and tissue degradation. Among these products are various cytokines, chemokines and tissue-degrading enzymes, such as the matrix metalloproteinases (MMPs), cell-surface molecules that enhance cell activation and cell–cell interactions, such as co-stimulatory and adhesion molecules like selectins, integrins, that are involved in inflammatory pathways creating important intercellular interactions pivotal in the inflammatory responses. Apart from the inflammatory infiltration in the peri-vascular and sublining regions of joints, the lining layer consisting of synoviocytes become hyperplastic, and transforms into an aggressive tissue at the cartilage–bone junction, the 'pannus', which contains osteoclasts which is responsible for major bone degradation. While degradation of cartilage is mainly mediated by a plethora of metalloproteinases present in the joint, bone destruction is mediated by the generation and activation of osteoclasts, an event that apparently does not occur in non-destructive arthritis [15, 16]. The role of B-cells and autoantibodies, and/or immune complexes may be important in the propagation and enhancement of the inflammatory process. B-cells function as effective antigen presenting cells and also produce the autoantibodies against RF citrullinated proteins [17]. A schematic presentation of the events taking place during RA is shown in the fig. 1.

A growing body of evidence indicates the possible role of highly reactive products of oxygen and nitrogen, termed as free radicals, in the pathogenesis of RA as well as other degenerative diseases [18]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced endogenously during aerobic metabolism at the sites of chronic inflammation. ROS such as superoxide radical, hydroxyl radical and hypochlorous acid contribute significantly to tissue injury in RA. In addition, activated leukocytes also produce ROS. Superoxide radicals and hydrogen peroxide do not directly damage the majority of biomolecules, but they are converted into the highly reactive hydroxyl radicals, which react with almost all molecules in living cells. ROS can directly or indirectly damage basic articular constituents and lead to the clinical expression of the inflammatory arthritis. Synovial cavity damage correlates with fluctuating oxygen pressure in the joint, over production of ROS, lack of oxygen-processing enzymes and free radical-scavenging molecules has been reported in RA. Oxidative stress exacerbates inflammation and worsens joint tissue.

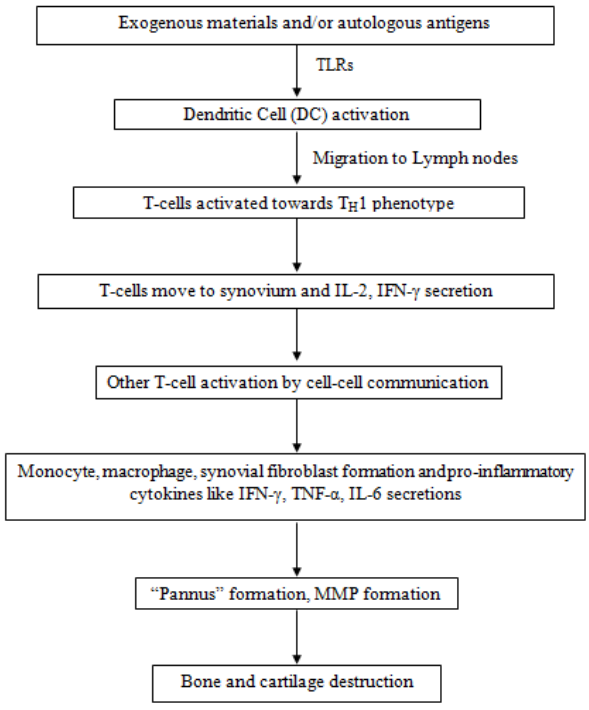

Fig. 1: Diagrammatic representation of the events of disease progression in RA

The normal equilibrium between ROS production and anti-oxidant system of the cell is disturbed due to oxidative stress, thus resulting in the damage to vital cell components such as proteins, DNA and membrane lipids. There are several studies demonstrating increased levels of malondialdehyde and decrease in the activities of catalase in RA patients. Similarly, glutathione reductase activities also get disturbed in the synovial fluid of patients. Moreover, the levels of thioredoxine, which is a marker of oxidative stress, are significantly higher in the synovial fluid of RA patients. Production of nitric oxide (NO) is also up regulated in arthritic tissue [18].

Genetic basis of rheumatoid arthritis

MHC and Rheumatoid arthritis

An extensive study of human genetics has been done to identify regions of the genome that are reproducibly associated with RA risk. Based on genetic information, from a large number of patients, the *HLA-DRB1* "shared epitope" alleles were first to be recognized in the late 1970's, followed by the discovery of *PTPN22* in 2004. In 2007, other three new loci have been identified (*STAT4*, *TRAF1-C5*, and *6q23*). Several other loci are showing promise, but the involvement of these loci with RA is not confirmed yet (*CD40*, *CTLA4*, and *PADI4*) [19].

The human leukocyte antigen (HLA) region remains one of the most powerful disease risk genes in different autoimmune diseases including RA. Several allelic variants of HLA-DRB1 genes have been associated with RA which supports a role for T-cell receptor-HLA-antigen to interact in the process. Disease-associated HLA-DRB1 alleles are polymorphic and certain allelic variants are preferentially high in the diseased population. Based on studies on the patients with the severe diseased condition a gene dose effect of HLA-DRB1 alleles has been suggested [20, 21]. Therefore, polymorphisms in HLA genes are being explored to find diagnostic tools for a rheumatoid syndrome. Besides HLA polymorphisms, other risk genes will be helpful in defining genotypic profiles correlating with disease phenotypes.

HLA genes in conjunction with other genetic determinants may lead the patient body to a certain pathway of synovial inflammation. Patients may or may not develop extra-articular manifestations, which are critical in determining morbidity and reducing healthy lifespan. HLA genes, complemented by other RA risk genes, are likely involved in shaping the T-cell repertoire. On the other hand, production of an unusual T-cell population characterized by the potential of vascular injury, as seen in extra-articular RA, is also accelerated by these genes [22].

Cytokines and rheumatoid arthritis

Cytokines are small polypeptide mediators of the immune and inflammatory responses. These molecules are secreted by almost all cell types at different titre in different time. Their action is generally at a paracrine or autocrine level. Cytokines function in a complex process with multiple relationships, making it difficult to predict the role of a single cytokine in any disease. The role of cytokines in the pathogenesis of RA has been the subject of multiple studies that generally agree on the overproduction of pro-inflammatory cytokines in the rheumatoid joint. There are several studies to monitor the roles of cytokines in different diseases. Most of these studies are based on immunohistochemistry or molecular biology techniques, which have detected the expression of cytokines (IL-α, IL-1b, IL-6, TNF-α, TGF-β) originating predominantly in the macrophage-fibroblast cells [23-26]. But it has been difficult to detect T-cell derived cytokines (mainly IL-2 and IL-4). However, these studies analyzed the synovial samples of patients with a long-standing disease treated with anti-rheumatic drugs which may alter the original profile of cytokine expression [27-29].

At present the mRNA expression of a broad spectrum of cytokines (IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, TNF-α, TGF-β) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been detected in the synovial tissues of RA patients. In both early and advanced stages of RA, it helps to know the cellular response and expression profiles of different cytokine molecules. The studies of the expression of HLA molecules during the progression of disease have been investigated to define precise cytokine–HLA network [27-29].

Role of cytokines in bone destruction

Studies using anti-TNF agents clearly show that they can slow down or prevent the progression of bone and cartilage damage in RA [30]. This activity probably involves suppression of osteoclasts-like cells in the joints [15]. Other cytokines like IL-1 and IL-17 are notable in regulating matrix degradation in animal models of arthritis [3]. The most exciting development in the pathogenesis of bone destruction in RA was the discovery of osteoclast-mediated bone resorption that is regulated by the RANK (receptor activator of nuclear factor (NF-kB) ligand or RANKL. RANKL is expressed by a variety of cell types involved in RA, including T-cells and synoviocytes. These cells, in the presence of cytokines like TNF-α and M-CSF, contribute to osteoclast maturation and activation. The soluble decoy receptor to RANKL, Osteoprotegerin (OPG), and RANKL are up regulated in RA, but normalize after treatment with TNF inhibitors [31]. The role of RANKL in inflammatory joint disease has been confirmed in several animal models. For instance, T-cell activation leads to an RANKL-mediated increase in osteoclasts and bone loss in rat adjuvant arthritis [32]. Osteoprotegerin administration to the arthritic animals blocks bone destruction but has a very little effect on inflammation. RANKL-knockout mice also have diminished bone erosion in arthritis models [33]. Cytokine pathways are involved in blocking the RANK/RANKL system that in the other hand blocks bone decay.

Mechanisms of cytokine gene expression and new therapeutic targets

As cytokines play a crucial role in any disease as a medium of cell-cell communication and cellular activation, cytokine regulation has been a key factor to regulate diseases like RA. For instance, NF-κB is activated in the synovium of patients with RA [34] which then regulates several genes, including TNF-α, IL-6, IL-8, inducible nitric oxidase synthase (iNOS) and cyclooxygenase-2 (COX-2), that contribute to inflammation. After stimulation of innate immunity or exposure to pro-inflammatory cytokines, the IκB kinase (IKK) signal complex is activated in synoviocytes, leading to phosphorylation of IκB [35]. IKKβ is both necessary and sufficient for induction of IL-6, IL-8 and intercellular adhesion molecule-1 (ICAM-1) gene expression. Targeting NF-κB is an effective therapeutic strategy in many animal models of arthritis. For instance, rat adjuvant-induced arthritis is suppressed by intra-articular gene therapy with dominant negative IKKβ adenoviral construct [36], while decoy oligonucleotides block streptococcal cell-wall arthritis [37].

The mitogen-activated protein kinases (MAPs) are also key regulators of cytokine and metalloproteinase production and could also be a target in RA. All three kinase families — extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 are expressed in rheumatoid synovial tissue [38]. All are constitutively expressed by cultured synoviocytes, and exposure to pro-inflammatory cytokines induces rapid phosphorylation. Upstream kinases that activate the MAP kinases, such as MKK3, MKK4, MKK6 and MKK7, are also activated in RA synovium and can form signalling complexes that integrate external environmental stresses to generate an appropriate cellular response. The MAP kinases have attracted considerable attention as potential therapeutic targets in RA. Pre-clinical studies show that p38 inhibitors are effective in murine collagen-induced arthritis, rat adjuvant-induced arthritis, and many other RA models [39]. A selective JNK inhibitor, SP600125, is mildly anti-inflammatory in the rat adjuvant-induced arthritis model but provides striking protection against bone and cartilage destruction [40]. Of the JNK isoforms, JNK2 is particularly important in arthritis because it is the dominant isoform expressed in synoviocytes. In JNK2-knockout mice, passive collagen-induced arthritis causes less cartilage damage compared to wild-type animals although clinical arthritis is still severe [41]. The transcription factor activator protein-1 (AP-1) also regulates many genes that participate in RA, including TNF-α and metalloproteinases.

High levels of AP-1 binding activity are detected in nuclear extracts of RA synovial tissue compared to osteoarthritis. Its components c-Jun and c-Fos are highly expressed in RA synovium, especially in the nuclei of cells in the intimal lining layer [42]. Pro-inflammatory cytokines can activate AP-1 activity in synoviocytes and lead to a

massive release of metalloproteinases. AP-1 molecules suppress collagen-induced arthritis and inhibit IL-1, IL-6, TNF-α, matrix metalloproteinase (MMP)-3 and MMP-9 production in synovial tissues [43]. One of the concerns with therapy directed at any of these key regulatory pathways is that they also participate in many normal cellular functions. The risks of toxicity or impaired host defence are significant potential problems because alterations in innate immunity or adaptive responses can be possible.

The advent of TNF inhibitors [44, 45] illustrates the success of applied translational research in RA, based on characterization of cytokine networks and studies suggesting that TNF-α production might serve as an autologous stimulus for other cytokines in RA synovium [46]. Although 40% of the patients have dramatic responses, the remainder have some evidence of persistent synovitis or minimal clinical benefit. IL-1Rα, a natural IL-1 antagonist, has also been approved for use in the United States of America. The response rates of IL-1Rα are less than that of TNF inhibitors, perhaps because IL-1Rα is a competitive antagonist that must be present in large excess. Additional cytokine-directed agents, such as anti-IL-6 receptor antibody, are also in clinical development, preliminary response rates being similar to TNF antagonists. Inhibition of IL-18 and IL-15 represents additional attractive approaches that could block T_H1 differentiation, production of inflammatory mediators, or TNF-α expression. Based on the T_H1 bias of T-cells in the synovium, treatment with T_H2 cytokines (IL-4, IL-10 and IL-13) was tested in many animal models of arthritis and has showed considerable promise [47]. But so far, administration of IL-10, which serves as a prototypic T_H2 cytokine, has met with only limited success [48].

Synthetic drug/plant extract & their efficacy in rheumatoid arthritis

There are two principal approaches to drug therapy for RA [4]:

- Symptomatic treatment with analgesics such as Acetaminophen and opioids, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), and intra-articular therapies such as glucocorticoids. These drugs only interfere with a small segment of the inflammatory cascade (e. g prostaglandin generation by cyclo-oxygenases [COXs]) but do not interfere with the underlying immuno-inflammatory events or retard joint destruction, and

- Disease-Modifying Anti-rheumatic Drugs (DMARDs) which 'modify' the disease process in all these respects, and once DMARDs are effective, no further symptomatic therapies are needed. Examples include methotrexate (MTX), sulfasalazine, leflunomide, hydroxychloroquine and few newer therapies such as anti-tumour necrosis factor (TNF)-α therapy (etanercept, infliximab and adalimumab), anti-CD20 therapy (rituximab) and abatacept.

All these NSAID and DMARD drugs are limited by low (<70%) response rate and induction (>30%) of severe adverse events. Some of the newer drugs are able to perform a little better in the remission rate and adverse events, but none of them have achieved ideal targets. This has been often cited as one of the reasons for increased use of complementary and alternative medicine (CAM) by patients suffering from RA. About 60% to 90% of patient populations have been reported to be using CAM from different countries [49].

According to the National Centre for Complementary and Alternative Medicine (NCCAM), "Complementary and alternative medicine is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine" [50].

The therapies and practices, included within the scope of complementary medicine field, can be broadly categorized into:

- Treatments in which people can administer themselves (e. g., botanical herbs, nutritional supplements, health food, meditation, and magnetic therapy),

- Treatments were administered by providers (e. g., acupuncture, chiropractic, massage, reflexology, and osteopathic manipulations), and

- Treatments people can administer under the periodic supervision of a provider (e. g., Tai Chi, yoga, homeopathy, and Ayurveda).

The world health organization (WHO) estimates that as many as 80% of the world population depends primarily on animal and plant based medicines. Of the 252 essential chemicals that have been selected by the WHO, 11.1% come from plants and 8.7% from the animals [50]. So there is an increasing need for research in this field.

Herbal medicine is the root of various traditional medicine systems around the world. Various traditional medicine systems around the world, including ancient Chinese and Indian medicinal system (consisting of two major branches—Unani and Ayurveda), rely heavily on herbs for health preservation and healing. Herbal medicines have been described in traditional texts and been used as anti-microbial, anti-inflammatory and anti-viral medicine for the cure of allergies, RA, infections, wound healing and fever. While many herbal extracts and formulations have undergone trials at different levels and have established their anti-arthritic potential, many need more research or remain partially explored.

At present, the current modalities for treating arthritis have not been shown to block, reverse or cure the disease. In these cases, disease flares up when the treatment is discontinued because the treatment is symptomatic. This has increased the interest of use of CAM therapies for the treatment of arthritis. However, much of the current research is focused on the identification, isolation and characterization of active principle(s) from crude extracts of known medicinal plants/herbs and animals/animal products, often overlooking the fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound and this may also help to nullify the toxic effects of individual constituents. The lack of sufficient clinical investigations too does not permit definitive conclusions to be drawn regarding the efficacy of plants and animals as CAM modalities in RA. However, several animal model based studies have helped in elucidating the potential mechanism of action of various CAM modalities. This encourages further studies but demands rigorous experimentation. There is a need for screening and scientifically evaluating number of known traditional medicinal plants and animals for providing newer and safer treatment options with minimum side effects. Once the underlying molecular mechanism for the observed anti-inflammatory and chondroprotective effects of nutraceuticals are elucidated, their health benefits may be fully exploited to develop new and better modalities for treating degenerative and inflammatory joint diseases.

Current knowledge on promising plant based medicines

***Camellia sinensis var. assamica* (J. W. Hart.) Kitam (Assam tea)**

Camellia sinensis (Family Theaceae) is native to east, south and south-east Asia, but it is today cultivated across the world in tropical and subtropical regions. The pharmacological properties of green tea are attributed to its high content of polyphenols/catechins, mainly epigallocatechin-3-gallate (EGCG). EGCG inhibits the transcription factor nuclear factor kappa-B (NF-κB), IL-1 induced phosphorylation of c-Jun-N-terminal kinase (JNK), expression and activities of matrix metalloproteinases MMP1 and MMP13 *in vitro* [51]. The catechin constituent of green tea was shown to inhibit the degradation of human cartilage proteoglycan and type II collagen. The effects of green tea were demonstrated in an animal model of inflammatory polyarthritis, wherein the collagen-induced arthritis (CIA) was ameliorated by prophylactic administration of green tea polyphenols (GTPs) in drinking water. In addition, the total immunoglobulins (IgG) and type II collagen levels were found to be lower in the serum and arthritic joints of GTP fed mice [18]. Immunostimulatory activity [52] and anti-microbial activity [53] of the plant is also promising.

***Curcuma longa* L. (Turmeric)**

Turmeric (Family-Zingiberaceae) is a commonly used colouring/flavoring agent with a long history of its use in Ayurveda for various medicinal conditions. It is native in south-east India and needs temperatures between 20-30 °C and a considerable amount of annual rainfall to thrive. The major component of turmeric is curcumin (diferuloylmethane) which constitutes approximately 90% of total curcuminoid content. Curcumin is a potent inhibitor of the common transcription factor NF-κB [54, 55]. Studies have also shown the inhibitory effect of curcumin on the arachidonic acid cascade (COX-2 and LOX) by inhibiting the catalytic activities of

phospholipases A2, Cγ1, and D. Curcumin also blocks the catabolic effects of IL-1 β induced upregulation of MMP-3, and IL-1β-induced decrease in type II collagen synthesis, that are known contributors in the pathogenesis of RA [56].

***Zingiber offcinale* roscoe (Ginger)**

Ginger (Family-Zingiberaceae) is indigenous to southern China, but now it is distributed worldwide and cultivated for different purposes. Ginger is a very commonly used dietary constituent worldwide, and is known to possess antioxidant, anti-inflammatory, antiseptic, and carminative properties. Ginger has a history of its use in Ayurveda for treating inflammatory and rheumatic diseases. The anti-inflammatory effects of ginger in treating arthritis are believed to be due to 6-gingerol, which is a pungent phenolic constituent of ginger. The 6-gingerol inhibits the LPS-induced NO production and effectively protects against peroxynitrite-mediated damage. Studies have shown that gingerols are excellent inhibitors of LPS-induced PGE2 production [57]. In some studies, RA patients experienced the marked reduction in pain after consumption of ginger [58, 59].

***Semecarpus anacardium* L. f. (Nut milk extract)**

Semecarpus anacardium (Family-Anacardiaceae) is a deciduous tree distributed in the sub-Himalayan tract and in tropical parts of India. In traditional medicine, it is highly valued for the treatment of gout, rheumatic pains, and cancer (60). The chemical constituents of the milk extract of *Semecarpus anacardium* include flavonoids, phenols, and carbohydrates. Studies have shown that *Semecarpus anacardium* or nut milk extract is effective against adjuvant arthritis (61**,** 62). The protective anti-oxidant role of flavonoids, is shown to be due to their inhibitory effects on the production of reactive oxygen species (ROS) by their free-radical quenching activities, and their potential to improve the levels of antioxidants in the body. Flavonoids have also been reported to exhibit anti-inflammatory activity by inhibition of phospholipase A2, thereby reducing the production of pro-inflammatory PGE2, and also by reducing the elevated levels of TNF-α and NO. Another protective effect of the plant is by enhancing the stability of the lysosomal membrane, thus preventing the rupture and release of lysosomal enzymes, which play a major role in erosive synovitis in RA (56). Furthermore, this plant has been shown to modulate both the humoral and cell-mediated immune responses along with its anti-inflammatory effects in adjuvant arthritic models. The humoral immunomodulatory response was explained by reversion of the elevated levels of IgG and IgA, and the cell-mediated immunomodulatory response by inhibition of T-lymphocyte migration to the inflamed joints.

***Saraca asoca* (Roxb.) Willd. (Ashoka)**

The original distribution of ***Saraca asoca*** (Family-Fabaceae) was in the central areas of the Deccan plateau of India, but now the plant is distributed throughout the country. Methanolic extracts of *Saraca asoca* has anti-inflammatory activity in rat arthritic models [63]. Treatment with *S. asoca* has also shown a significant reduction in the levels of both plasma and liver lysosomal enzymes. The protein bound carbohydrates and urinary collagen contents were also decreased at a significant level by the treatment of *S. asoca* methanol extract. Furthermore, treatment of *S. asoca* reduced the levels of pro-inflammatory cytokines in adjuvant-induced arthritic rats [64].

***Ananas comosus* (L.) Merr. (Pineapple)**

Ananas comosus (Family-Bromeliaceae), commonly known as Pineapple, is native to Central and South America and is grown in several tropical and subtropical countries including the Indian subcontinent. Chloroform and methanolic extracts of *A. comosus* leaf have shown activity against acute anti-inflammation in carrageenan-induced paw oedema in Wistar albino rats. The methanolic extract was found to be the most potent followed by the chloroform extract [65]. Bromelain, a protease extracted from the stem of pineapple reduces mild acute knee pain [66].

***Cannabis sativa* L. (Ganja)**

This species belonging to Family Cannabaceae is indigenous to Central and South Asia. Cannabis is one of the oldest known medicinal plants and produces pharmacologically important substances. Among them, most important are the cannabinoids that are unique components in the cannabis plant. The 9-Tetrahydrocannabinol (9-THC) and cannabidiol (CBD) are known to be major cannabinoids in the plant. Cannabidiol (CBD), a non-psychoactive marijuana constituent, was recently shown to act as an oral anti-hyperalgesic compound in a rat model of acute inflammation [67]. Cannabidiolic acid was shown to be a selective cyclo-oxygenase-2 (Cox-2) inhibitory component in cannabis [68].

Cannabinoids mediate their physiological and behavioral effects by activating specific cannabinoid receptors. With the recent discovery of the cannabinoid receptors (CB1 and CB2) and the endocannabinoid system, research in this field has expanded exponentially. Cannabinoids have been shown to act as potent immunosuppressive and anti-inflammatory agents and have been shown to mediate the beneficial effects in a wide range of immune-mediated diseases such as multiple sclerosis, diabetes, septic shock, rheumatoid arthritis, and allergic asthma. Cannabinoid receptor 1 (CB1) is mainly expressed on the cells of the central nervous system as well as in the periphery. In contrast, the cannabinoid receptor 2 (CB2) is predominantly expressed on immune cells [69].

***Ocimum tenuiflorum* L. Syn. *Ocimum sanctum* L. (Tulsi)**

Ocimum sanctum Linn. (Family-Lamiaceae), a small herb seen throughout warmer parts of India, have been recommended by Ayurveda for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The fixed oil (non-volatile part of the plant, typically obtained from the seed or nut) of *O. sanctum* was shown to have anti-arthritic activity in Freund's adjuvant-induced arthritis, formaldehyde-induced arthritis and also in turpentine oil-induced joint edema in rats. The fixed oil showed significant anti-arthritic activities in both models and anti-edema activity against turpentine oil-induced joint edema [70]. The oil shows anti-inflammatory potential due to inhibition of arachidonate metabolism and anti-histaminic activity. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum* L., has been found to be largely responsible for its therapeutic potential.

***Withania somnifera* (L.) Dunal *(*Ashwagandha)**

Withania somnifera (Family-Solanaceae) is a small herb found in different parts of India and Nepal. It has promising anti-arthritic activity, ascribed to its stabilizing action on lysosomal enzyme activities [71]. The aqueous suspension of *Withania somnifera* root powder showed potent inhibitory activity towards the complement system, mitogen-induced lymphocyte proliferation and delayed-type hypersensitivity reaction. Immunosuppressive effect of *W. somnifera* root powder indicates that it could be a candidate for an immunosuppressive drug for inflammatory diseases [72]. Oral administration of *W. somnifera* root powder showed a significant increase in the level of lipid peroxides, glycoproteins, and urinary constituents with the depletion of antioxidant status and bone collagen in arthritic animals [73]. The effect of *W. somnifera* crude ethanol extract was studied on peripheral blood mononuclear cells of normal individuals and RA patients and synovial fluid mononuclear cells of RA patients *in vitro.* The study demonstrated that crude ethanol extracts of *W. somniferra* suppressed the production of pro-inflammatory molecules *in vitro*. This activity is partly through the inhibition of transcription factors NF-κB and AP-1 by the constituent with anolide [74]. Withaferin-A, a steroidal lactone from *W. somnifera* shows potent anti-arthritic and anti-inflammatory activity in arthritic animals [75].

***Moringa oleifera* Lam. (Moringa)**

Moringa oleifera (Family-Moringaceae), commonly known as moringa, drumstick, and horseradish, is a small, fast-growing tree that originates in India. Ethanolic seed extracts of *Moringa oliefera* is shown to have immunosuppressive activity in experimental immune inflammation [76]. A rare aurantiamide acetate and 1,3-dibenzyl urea has been isolated from the roots of M. oleifera [77]. In a study, the anti-arthritic activity of a hydro-alcoholic extract of Moringa flowers was investigated in adjuvant-induced arthritis in Wistar rats. Body weight, paw edema volume (primary lesion), inflammation at non-injected sites, and arthritic index (secondary lesion) in diseased

animals was reduced by treatment with the extract as compared to untreated control animals. The protective effects of Moringa were also noted in serum levels of Rheumatoid Factor (RF) and levels of the cytokines, TNF-α and IL-1 in treated diseased animals as compared with untreated control animals. Test animals showed decreased RF level, TNF-α and IL-2 levels in the serum when treated with Moringa. Histopathological sections from animals in the drug treatment group showed a protective effect that was evidenced by less infiltration of lymphocytes and less angiogenesis as compared with sections from arthritic animals [76].

***Cyanthillium cineveum* (L.) H. Rob. Syn. *Vernonia cinerea* (L.) Less (Sahadevi)**

This plant is very common in West and Central Africa but well distributed in India as well. It belongs to the Family Asteraceae. Latha *et al.* (1998) [78] tested the anti-inflammatory effect of an alcoholic extract from the flower of *Cyanthillium cineveum* (Syn. *Vernonia cinerea*) (Family-Asteraceae) in adjuvant arthritic rats. Changes in paw volume, body and tissue weights and serum and tissue enzyme activities of ALT, AST, ACP and cathepsin-D in adjuvant rats were reversed by oral administration of 100 mg/kg body weight of the flower extract. The extract also reversed the major histopathological changes in the hind paws of the arthritic rats. Phytochemical studies revealed the presence of alkaloids, saponins, steroids and flavonoids [78].

Methanolic extract of *Cyanthillium cineveum* was found to scavenge the hydroxyl radical generated by Fenton reaction, superoxides generated by photo- reduction of riboflavin and to inhibit lipid peroxidation significantly. The drug also scavenged nitric oxide. Intra-peritoneal administration of *Cyanthillium cineveum* was found to inhibit the PMA induced superoxide generation in mice peritoneal macrophages. The administration of *Cyanthillium cineveum* to mice significantly increased the levels of catalase, superoxide dismutase, glutathione, glutathione peroxidase and glutathione-S transferase in blood and liver, whereas lipid peroxidation activity was significantly decreased [78]. It was also found that *Cyanthillium cineveum* extract significantly inhibited carrageenan-induced inflammation, compared to control models. Down regulation of pro-inflammatory cytokine level and gene expression data also supported the above results [79].

***Justicia gendarussa* Burm f. (Water willow)**

Justicia gendarussa (Family-Acanthaceae) is a shade-loving, quick-growing, evergreen plant mostly found in moist areas. It is believed to be native to China and is distributed widely across India, Sri Lanka, and Malaysia. In Indian and Chinese traditional medicine, the leaf of the plant is recommended to treat ailments such as fever, hemiplegia, rheumatism, arthritis, headache, ear ache, muscle pain, respiratory and digestive disorders. The paw volume and lipid peroxide level of hemolysate and liver in arthritic rats were significantly reduced to near normal level by administration of ethanolic leaf extract of *Justicia gendarussa* [80]. The decreased level of enzymatic antioxidants activities and non-enzymatic antioxidants levels was reverted to normal levels when methanolic extract given to arthritic rats. The preliminary phytochemical analysis of methanolic extract showed the presence of many biologically active phytochemicals such as flavonoids, alkaloids, phenolic compounds, saponins, glycosides, and tannins and these compounds might be responsible for the anti-inflammatory properties. The possible anti-inflammatory mechanism of the *Justicia gendarussa* leaf extracts may be through its free radical scavenging activity, its stabilizing action on lipid peroxide and increased antioxidants levels [81].

Another study focused on the anti-inflammatory activity of *Justicia gendarussa* in a carrageenan-induced paw edema assay. Methanolic extract of *J. gendarussa* (JRM) roots significantly inhibited oedema formation 5 hours after carrageenan induction. JRM inhibited carrageenan-induced change in total cycloxygenase activity; 5-lipoxygenase and 15-lipoxygenase activities in blood mononuclear cells of rats, decreased neutrophil infiltration in paw tissue as shown by low myeloperoxidase activity [82]. It also caused an inhibition in" with "inhibited cyclooxygenase-2 activity in paw tissue [82]. Purification of JRM by liquid–liquid partitioning yielded an ethyl acetate fraction of JRM that showed interleukin-6 downregulation potential and the ability to inhibit prostaglandin E2 production *in vivo* [82].

***Premna serratifolia* L. (Agnimantha)**

Premna serratifolia (Family-Lamiaceae) is a large shrub widespread in the deciduous forests of India. The whole plant possesses medicinal properties, useful in the treatment of cardiovascular, skin, inflammatory diseases, arthritis, gonorrhoea, rheumatism, anorexia and jaundice. The anti-arthritic activity of ethanolic extract of *Premna serratifolia* wood was tested in Freund's adjuvant-induced rat arthritis model. The loss in body weight during arthritis condition was corrected on treatment with ethanol extract and standard drug indomethacin [82]. Biochemical parameters such as hemoglobin content, total WBC, RBC, erythrocyte and sedimentation rate were also estimated. The ethanolic extract at the dose of 300 mg/kg body weight inhibited the rat paw edema by 68.32% which is comparable to standard drug indomethacin which inhibited 74.87% paw edema after 21 days. The observed anti-arthritic activity may be due to the presence of phytoconstituents such as irridiod glycosides, alkaloids, phenolic compounds and flavonoids [83].

***Cissampelos pareira* L. (Abuta)**

Cissampelos pareira (Family-Menispermaceae) is a common plant seen in the subcontinent. In a study, 50% ethanolic extract of C. the roots exhibited significant anti-inflammatory activity in acute, subacute and chronic models of inflammation in rats [84]. In the same study, 50% aqueous ethanolic extract of *C. pariera* exhibited resistance against pain in mice. Further, it also showed resistance against adjuvant-induced arthritis in mice [84].

***Nyctanthes arbor-tristis* L. (Harshringar; Shiuli)**

Harshringar or *Nyctanthes arbor-tristis* (Family-Oleaceae) has been used widely as a decoction for the treatment of arthritis and sciatica in the Indian ayurvedic system of medicine for centuries. The plant has its origin from the Bengal region of India while it is distributed all over sub-tropical regions of the country. Arbortristosides, nyctanthic acid, and crocetin are the main active principles of the plant. Water soluble ethanolic leaf extract has been reported to reduce significantly the levels of inflammatory cytokines (IL-1, TNF-α) in experimental arthritis [85].

***Swertia chirayita* (Roxb.) Buch-Ham. Ex C. B. Clarke (Chirata)**

Swertia chirayita, (Family-Gentianaceae) a herb found abundantly in the temperate regions of Himalaya, is commonly used for chronic fever, anemia and asthma. The plant inhabits the pastures and slopes of the Himalayas and ranges between 2000 to 3,000 metres. Chirayita comprises of swerchirin, swertanone and swertianin as active components responsible for the anti-inflammatory activity. Chirayita is reported to reduce the elevated levels of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in experimental arthritis as well as in asthmatic conditions [86].

***Crocus sativus* L. (Saffron)**

Saffron (Family-Iridaceae) is native to Greece and south-west Asia and it was brought to this country and naturalized later. The plant is commonly used as folk medicine for various purposes such as aphrodisiac, anti-spasmodic and expectorant. It is a perennial flowering plant with very less growing height up to 40 cm. Commonly cultivated in Kashmir, the distribution of the plant is seen in North America, Greece and Spain as well.

Saffron stigma possesses anti-inflammatory action due to presence of the crocetin and carotenoids. Aqueous and ethanolic extracts of saffron petals exhibit radical scavenging as well as anti-inflammatory effects in xylene and formalin-induced inflammation [87].

***Strobilanthus callosus* Nees. (Karvi)**

Karvi (Family-Acanthaceae) is another Indian medicinal herb, commonly found in the Maharastra state and has been used by the local tribals for the treatment of inflammatory disorders. The Lupeol and 19α-H Lupeol isolated from the roots of *Strobilanthus callosus* have demonstrated the anti-inflammatory as well as anti-rheumatic activity in carrageenan-induced oedema [88].

***Aloe vera* (L.) Burm. f. (Ghritokumari)**

Dermatological aspects of *Aloe vera* (Family-Xanthorrhoeaceae) are very well known in the traditional medicinal systems. In this regard, studies on compound isolation are also done revealing the presence of potent anti-inflammatory biomolecules in the plant [89]. The plant is well distributed in India and neighbouring countries. Anti-inflammatory activity has been investigated in the *Aloe vera* crude extract in carrageenan-induced arthritic rat models [90]. Preliminary studies on anti-arthritic activity have also been done [91]. Experiments prove that the plant has some strong protective role against inflammation and rheumatism. However, some more precise experiments are needed to establish its role in such disease.

Other important plants with anti-inflammatory activities

Acacia farnesiana (L.) Willd. [92], *Aegle marmelos* (L.) Correa (Bel) [93], *Anacardium occidentale* L. (Cashew) [94], *Azadirachta indica* A. Juss. (Neem) [95], *Cedrus deodara* (Roxb. ex D. Don) G. Don (Deodar) [96], *Morus indica* L. (Mulberry) [97], *Emilia sonchifolia* (L.) DC. Ex DC.[98] are some other promising plants having potential anti-inflammatory activities. These plants can be further used to investigate their role in rheumatoid arthritis.

***Saraca asoka* (Ashok)** | ***Cannabis sativa* (Ganja)** | ***Withania somnifera* (Ashwagandha)**

***Cissampelos pereira* (Abuta)** | ***Justcia gendarussa* (Water willow)** | ***Aloe vera* (Ghritokumari)**

Fig. 2: Promising medicinal plants of the North-Eastern Himalayan region having anti-arthritic and anti-inflammatory properties. Leaf, fruiting bodies and flowers are shown in insets. (Photo courtesy: Garden of Medicinal Plants, University of North Bengal, India)

CONCLUSION

At present, the current modalities for treating arthritis are symptomatic and have not been shown to either block or reverse the cartilage degradation and joint destruction. This has resulted in heightened interest in the use of CAM therapies for the treatment of arthritis. However, much of the current research is focused on the identification, isolation and characterization of active principle(s) from crude extracts of known medicinal plants/herbs and animals/animal products, often overlooking the fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound and this may also help to nullify the toxic effects of individual constituents. Also, the lack of sufficient clinical investigations does not permit definitive conclusions to be drawn regarding the efficacy of plants and animals as CAM modalities in RA. However, several animal studies have helped elucidate the potential mechanism of action of various CAM modalities. This encourages further studies but demands rigorous experimentation. There is a need for screening and scientifically evaluating number of known traditional medicinal plants and animals for providing newer and safer treatment options with minimum side effects. Once the underlying molecular mechanism for the observed anti-inflammatory and chondro-protective effects of nutraceuticals are elucidated, their health benefits may be exploited to develop new and better modalities for treating degenerative and inflammatory joint diseases.

CONFLICT OF INTERESTS

Declared None

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