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***Review Article***

# Ethno biological usage of zoo products in rheumatoid arthritis

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Rheumatoid arthritis (RA) is one of the most common autoimmune disorder which causes swelling, redness, pain, stiffness, restriction of limb movements, decreases life expectancy and early death of the patients. Available drugs include non steroidal anti-inflammatory and analgesics, disease modifying anti-rheumatic drugs and steroids (glucocorticoids etc). All these drugs have their own limitations such as gastrointestinal irritations, cardiovascular problems, and drug dependency. Search for alternative therapy from natural products are being ventured throughout the world. Zoo therapy in arthritis, a common practice of the ancient times that have been mentioned in traditional and folk medicine. The scientific basis of some of the zoo products are being explored and have been showing promising results in experimental rheumatoid arthritis. These therapies have minimum side effects and many of them have potential to give rise to drug development clues against rheumatoid arthritis. The present review is an effort to establish the folk and traditional treatment of rheumatoid arthritis using zoo products.

**Keywords:** Alternative medicine, Rheumatoid arthritis, Traditional medicine, Zoo products

Arthritis is a general term used to describe many connective tissue disorders that affect bone and joints. The word arthritis came from Greek word '*arthon*' means joints and '*itis*' means inflammation. In 85,000,000 BC, secondary osteoarthritis was found in ankle joint of dinosaurs and in 28,000 BC, Neanderthal man showed signs of secondary osteoarthritis. The first evidence for arthritis in man came from 4500 B.C[1]. Otzy, a frozen body in Alps found with a bag full of herbs, had osteoarthritic problems in his knee. Arthritis has been mentioned in the ancient Hindu and Greek mythology[2]. The first written reference on arthritis was found in Indian holistic medicinal book Charaka Samhita, where it was described as swollen painful joints, initially occurring in the hands, feet, causing loss of appetite and occasionally related with fever[3]. Arthritic symptoms were also found in Europe, where in 13$^{th}$ century any joint problems were termed as "gutta" or gout[4]. There are hundred types of arthritic conditions, which could be classified under three major categories—rheumatoid arthritis, osteoarthritis and gouty arthritis.

## Rheumatoid arthritis (RA)

The term rheumatoid arthritis was first coined by Garrod in 1859[5]. It is an autoimmune disease with chronic inflammation, characterized by pain, redness and swelling of the affected joints, stiffness of the surrounding muscles which ultimately leads to the destruction of the cartilage and bone with substantial loss of functioning and mobility. In fact it is the most common autoimmune disorder in the world, affecting nearly about 1% of the population[6]. Both sexes are

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affected by the disease, but females are more susceptible, approximately 3:1 ratio in favor of females[7]. The onset of the disease is insidious, beginning with prodrome of fatigue, weakness, joint stiffness, vague arthalgias and myalgia, followed by pain, swelling of joints usually in symmetrical fashion. Its systemic manifestation includes haematological, pulmonary, neurological and cardiovascular abnormality. The symptoms of rheumatoid arthritis also include rheumatic fever, sclerosis, bow leg and nodule formation[8].

**Pathophysiology of RA**

The etiology and pathophysiology of RA is not fully understood yet. Autoimmune and genetic factors are involved in the disease. The immunological events are thought to be the basis of RA development. Individual with rheumatoid arthritis produce a group of antibodies called rheumatoid factors which is autoantibody that recognizes Fc region of IgG[9]. Such autoantibodies bind to normal circulating IgG with classic rheumatoid factor IgM, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a type III hypersensitivity reaction, which leads to chronic inflammation of the joints[10]. Sometimes, autoantibody like antinuclear factor or type II collagen autoantibody was also associated with this disease. CD4+ T cell plays a key role in this process[11]. Exogenous or endogenous factors are presented to the CD4+ T cells. T cells get activated and stimulates monocytes, macrophages and synovial fibroblasts to produce inflammatory cytokines causing leukocytes infiltration and augment inflammatory cascade[12]. The interleukins also activate fibroblasts, which release matrix metalloproteinase causing erosion of bone and joints. Arachidonic acid metabolism in macrophage increases during this pathological condition and induces cyclooxygenase (COX) activation and prostaglandin production, which cause pain, fever and inflammation[13]. Angiogenesis is also promoted by activated macrophage, lymphocyte, and fibroblast or by their products. This increases the vascularity of the joints in RA patients. The activated T cell activates the osteoprotegerin ligand, which stimulates osteoclastogenesis there by affecting bone turnover[14]. Endothelial cells which are also activated by T cells secrete adhesion molecules that help in the recruitment of more inflammatory cells into the affected joints[12].

Genetic role behind the disease is not fully explored. Familial aggregation and disease concordance in twins were the first hints for the contribution of genetic factors involved in the RA susceptibility. This familial aggregation frequency is very low[15]. It first drew attention to the relation of RA with one HLA-D type, Dw4 in Caucasian patients[16]. It had been studied that concordance rate was only 12-15% in identical monozygotic twins[17]. However, human leukocyte antigen (HLA) association supports the hypothesis that genetic factors are important for RA susceptibility.

**Therapeutic managements of RA**

The aim of the management of rheumatism is to reduce pain and to minimize the changes which occur during RA development. Physiotherapy, physical exercise and analgesics are often prescribed by rheumatologists. Non steroidal anti-inflammatory drugs (NSAIDs) are the first line of defence against arthritis, which include aceclofenac, diclophenac etc. In 1763, willow bark, which contains salicin (later known as acetyl salicylic acid) a NSAID, was first introduced to treat rheumatism and arthritis. Aspirin and other NSAIDs work through cyclooxygenases (COX1 and COX2) that inhibits prostaglandin biosynthesis[18]. COX1 inhibition causes side effects like gastrointestinal (GI) irritation, platelet aggregations etc. Rofecoxib, cellecoxibs can effectively block COX2 without causing GI irritations. Cardio vascular problems may arise with coxib treated patients, as it inhibits prostaglandins (PG) biosynthesis in the vessel wall[19]. The COX inhibitions help to protect against arthritic inflammation, pain, and rheumatic fever. Steroids, mainly glucocorticoids are used in the treatment of arthritis and rheumatisms[20], where only 30% patients do not respond to this treatment. There are several other side effects associated with glucocorticoid therapy, such as immune suppression, steroid induced osteoporosis, muscular breakdown, pubertal delay etc.[21] Therefore with more realistic aims to alleviate pain, suppress inflammations, prevent joint damages and loss of joint functions, disease modifying anti rheumatic drugs (DMARDs) are better advice[22]. Drugs like methotrexate or cyclosporine A or anti-cytokine therapies should be applied early in the course of disease though they also have side effects. Anti-tumour necrosis factor (Anti-TNF) therapy with infliximab increases the chance of infections. Sepsis, pulmonary and extra pulmonary tuberculosis and

other opportunistic infections increase after this treatment[23]. Glomerulonephritis may develop through rheumatoid arthritic-related nephropathy in rheumatoid arthritic patients receiving anti-TNF-α agents[24]. Other therapeutic interventions include B-cell depleting agent (rituximab), IL-1 receptor antagonist (anakinra), intramuscular gold, immunomodulatory and cytotoxic agents (azathioprine, cyclophosphamide, cyclosporine A).

**Zoo products active against RA**

Indian traditional medicines which include ayurveda, homeopathy, unani and siddha are dependent on the usage of several natural products. In Ayurveda "Bhasma" of different animals (cone shell, coral calx, sankha bhasma, kapardika bhasma, etc) are used against arthritis[25]. In Unani, natural products are used to treat pain related to arthritis. Animals / animal products are used in homeopathy drug such as snake venom, bee venom etc. Magical, rituals, religious practice are also involved in zoo medicine[26]. In Santhal medicine, several animal products (horn, bone, dung, etc) are used along with mantra, chanting, etc[27]. WHO had selected 252 essential chemicals as medicine for respiratory, rheumatic pain, skin related problems, gastro intestinal disorders, eye and ear related problem and 8.7 % of these are from animals[28]. Out of the 150 prescribed drugs against several disorders, 27 were of animal origin[29]. In different parts of India, 270 varieties of uses from nearly 109 animals and had been reported in the traditional medicine. In rheumatoid arthritis and other pain related problem, 34 uses from 32 species have been mentioned[30].

The phenomenon of zoo-therapy depends both on geographical distribution and historical origin[31]. In Sudanese traditional medicine, the fresh manure of a dromedary (*Camelus dromedaries*) is used topically to alleviate arthritis. Hyana (*Hyaena hyaena*) fat was also used in Sudan to treat arthritis and rheumatism[32]. Most African people believe that animals and their bi-products have some magical powers attached which actually help in the healing processes. In Nigeria, fat extracted from manatee (*Tricheus senegalensis*) is used to treat this disease[33]; where as in south western Nigeria, snail shells (*Archanchantina maginata*) are broken and the snail fluid is immediately swallowed, which helps in treating rheumatism[34]. In Brazil, fats and oils from different animals have been used to alleviate arthritis and rheumatism. Sharp nose shark (*Rhizoprionodon porosus*), nurse shark (*Ginglymostoma cirratum*), green turtle (*Chelonia mydas)*, the hawksbill turtle (*Eretmochelys imbricata*), the loggerhead turtle (*Caretta caretta*), the leatherback turtle (*Dermochelys coriacea*), electric eel (*Electrophorus electricus*) and Amazon river dolphin are used to treat rheumatisms and arthritis[35]. The fat snakes like Rattlesnake (*Crotalus durissus*), Boa (*Boa constrictor*), Rainbow boa (*Epicrates cenchria*), Anaconda (*Eunectes murinus*) have been used in the treatment of rheumatoid arthritis[36]. Minke whale (*Balaenoptera acutorostrata*) and Sheep (*Ovis aries*) fat have been used in Brazil for the same cause. The body fat of *Tupinambis merianae* demonstrated significant topical anti-inflammatory activity in experimental animal model[37]. Leeches have been used traditionally to alleviate abnormal swelling, pain and arthritis[38]. In Korea and China, animals are used traditionally to treat different diseases. Insects have been involved in preparing many alternative medicines including medicine for rheumatism[39]. Bee sting is considered useful in treating arthritis and related pain. Honey, a by product of bee, mixed with luke warm water and small teaspoon of cinnamon powder (Kanafuru) when rubbed on the affected area of the body, reduced pain quickly[34]. The centipedes with their numerous legs, feet, and articulated body segments have been used for leg foot and joint problems. Scorpion sting that produces pain, could be used to treat pains of different kinds[40]. In Chinese medicinal system, bones of tiger (*Panthera tigris*) are often boiled and the concentrate is used to relieve pain and arthritis related problems. The monkey skeleton is used to treat general pain, arthritis related pain.

In Indian holistic traditional medicinal practice "Charaka Samhita", approximately 380 types of animal based medication are used which include animal parts, products and processed products. Many of the animals have been used for generations and have been incorporated in Ayurveda. Approximately 15 to 20% of Ayurvedic drugs used, are of animal based[41]. Venom is a major part of zoo-therapeutic approaches from ancient times. In Indian traditional medicinal practice of Ayurveda, venoms were used to treat Dushyodara, Jalodara (ascitis), sannipatik jwara as hepatic stimulant and most importantly to treat arthritis and related pain[42,43]. Suchika Voron (venom at the tip of a needle) was a common practice in treating rheumatism. Cobra venom (*Naja kaouthia, Naja naja*) and shodhita (detoxified) cobra venom have been used to treat arthritis and related pain[44]. In

santhal medicine, animals are used frequently in treating different diseases including arthritis[27]. In the tribal villages of India, scorpions were dipped into mustard oil and were used tropically to treat arthritis, pain and inflammation. People of Kosi river basin of North Bihar, India prepare a soup with the foot pad of fresh water snail (*Bellamia bengalensis*) and use to cure arthritis and rheumatism[45] (Table 1).

**Zoo product derived active constituents against RA**

Epidemiological studies showed that the native people of Greenland, Japan and the Eskimos have lesser evidence of autoimmune diseases like rheumatoid arthritis as compared to the Europeans[46]. The reduced autoimmunity was probably due to their consumption of fish diet[47]. In traditional medicine, animal fats and oils (fish oil, cod liver oil, shark oil) were used in the treatment of arthritis and have been tested for their anti-arthritic activities. Fish and cod liver oil possessed a large amount of $\omega^3$ fatty acids (eicosapentanoic acids and decosahexanoic acid) which help to reduce arthritic conditions[48]. Consumption of fish oil increased concentration of $\omega^3$ fatty acid in all the cells including monocytes, macrophages and poly morphonuclear (PMN) cells compete with $\omega^6$fatty acids (arachidonic acid) in the membrane. In PMN cells, eicosapentanoic acid replaces arachidonic acid and thereby converted into the biologically less active prostaglandin E3 (PGE3) and leukotriene B5 (LTB5) instead of PGE2 and LTB4 formation[49]. Eicosapentanoic acid (EPA) and decosahexanoic acid (DHA) present in fish oil, decrease PGE2 formation. It is well known that PGs play an important role in immunity and inflammation[50]. When the PG profile of inflammatory cells is altered by a fish oil diet, these cells show functional changes that tend to reduce inflammation[49, 51]. It has been suggested that patients with rheumatoid arthritis can be benefited by an EPA-enriched diet[48,52].

The effect of fish oil in cytokine production is contradictory. In human studies, *ex vivo* production of IL-1, IL-6 and TNF-α from peripheral mononuclear cell were decreased after supplementation of diet with $\omega^3$ fatty acids[53,54]. There were no differences in *ex vivo* stimulated IL-Iβ production in aged human subjects after four months of dietary fish oil supplementation[55]. Decrease *in vitro* production of IL-1 and TNF-α by liver macrophage of rats after six weeks of fish oil supplementation have been reported[56]. The synthesis of IL-1 and TNF was studied by mouse peritoneal macrophages[57]. Fish oil supplementation for four weeks showed increased IL-1 and TNF production. Enhanced *in vitro* stimulated TNF production by resident peritoneal macrophages in $\omega^3$ fatty acid fed mice have been observed[58]. Thus the effects of dietary fatty acids on cytokine production in mice were opposite to those in human and rats.

*In vitro* addition of PGE2 to human peripheral blood mononuclear cells inhibited TNF production, probably by increasing intracellular cyclic AMP[59,60]. Initially, it was found that, *in vitro* production of IL-1 was inhibited by PGE2, but since the availability of specific IL-1 assays, it has become clear that *in vitro* IL-1 secretion was not inhibited by PGE2[59,61]. Inhibition of PGE2 production *in vitro* by cyclooxygenase inhibitor has been reported to increase circulating concentrations of IL-6 and TNF in humans[62]. As consumption of a $\omega^3$ fatty acid-rich

Table 1—Zoo product in practice against arthritis

| Animal | Portion use | Composition | Probable mechanism | Reference |
|---|---|---|---|---|
| Ant | Venom | Protein/ peptide | Inflammatory modulators | 73, 74 |
| Bee | Venom | Protein/Peptide | Inflammatory modulators | 75, 76 |
| Scorpion | Venom | Protein/ peptide | Inflammatory modulators | 84 |
| Snail | Fluid and foot pad | Not Known | Inflammatory modulators | Unpublished |
| Mussel | Whole body | $\omega^3$ fatty acids | Inflammatory modulators | 64, 65 |
| Shark | Fat | $\omega^3$ fatty acids | Prostaglandin blocker | - |
| Fish | Fats / Oils | $\omega^3$ fatty acids | Prostaglandin blocker | 48 |
| Turtle | Fat | $\omega^3$ fatty acids | Prostaglandin blocker | - |
| Snake | Fat | $\omega^3$ fatty acids | Prostaglandin blocker | - |
| Cobra | Venom | Protein/ peptide | Inflammatory modulators | 81 |
| Manatee | Fat | $\omega^3$ fatty acids | Prostaglandin blocker | 71, 33 |
| Dromedary Camel | Manure | Not known | - | - |
| Hyana | Fat | $\omega^3$ fatty acids | Prostaglandin blocker | 32 |
| Tiger | Bony skeleton | Not known | - | - |

diet decreases PGE2 concentrations, it is unlikely that prostaglandins play an important role in the decreased TNF production observed in humans and some other species. After the abolition of PGE2 production by indomethacin, peritoneal macrophages of fish oil fed mice still produced significantly more TNF[58]. This indicated that in mice, reduced PGE2 production was not the only mechanism responsible for the increased TNF production. A reduction in plasma IL-1β concentrations had been reported after fish oil supplementation in rheumatoid arthritis patients[63].

New Zealand green lipped mussel *Perna cannaliculas* is known to contain anti-inflammatory activity[64,65]. Feeding with *P. cannaliculas* improved arthritic signs in dogs[66]. Lyprinol, a patented extract from *P. cannaliculas* was reported to be very effective and promising anti-inflammatory product that relieved the signs and symptoms of osteoarthritis, without adverse effect[67]. The lipid extract of *P. cannaliculas* was effectively inhibited 5/ lypooxygenase and cyclooxygenase pathways involved in the production of eicosanoids, including leukotriens and prostaglandins[68]. *Perna cannaliculas* inhibited experimentally induced inflammation. The activity was thought to reside within aqueous fraction containing high molecular weight material, possibly a polysaccharide glycogen. This glycogen administration results dose dependent anti-inflammatory effects in rats with carrageenin induced footpad edema. Mobilization of neutrophils to the site of an inflammatory stimulus was also significantly reduced. However this activity was lost, if the glycogen extract was treated with KOH or proteinase K, suggesting that the anti-inflammatory properties resides within a protein moiety associated with glycogen[69].

Indian fresh water edible snail (*Bellamia bengalensis*) extract showed anti arthritic activity in adjuvant induced arthritic rat models. In *Bellamia bengalensis* extract treated experimental rats paw and ankle diameters, paw weight, arthritic nodules formation, hydroxyproline, glucosamine levels were significantly decreased compared to untreated control arthritic group of rats. Snail extract possessed anti-inflammatory activity and gave protection against oxidative damage in experimental adjuvant induced rat models by restoring serum acid phosphatase, alkaline phosphatase, IL-6, CINC1, IL-10 and TNF-α levels. Erosion of cartilage and ruptured synovial membrane in knee joint of arthritis animal were partially restored in *B. bengalensis* extract treated experimental rats (unpublished data). Anti-arthritic activity has been reported from Indian fresh water mussel *Lamellidens marginalis*[70], the aqueous extract (per oral) significantly decreased paw diameter, ankle diameter and paw weight in Freund's complete adjuvant (FCA) induced arthritis in experimental animal model. The extract significantly restored urinary hydroxiproline, glucosamine level, serum IL-1β, IL-6, CINC1, TNF-α, IL-10 and lysosomal enzyme level. Synovial membrane damage and neutrophil infiltration in histopathological examination were restored significantly with this *L. marginalis* aqueous extract.

The Indian Monocellate Cobra (*Naja kaouthia*) venom (NKV) showed anti-arthritic activity over FCA induced arthritis in male albino rats[71]. NKV treatment (1/20th and 1/10th minimum lethal dose (MLD) doses for 13 days, i,p.) showed significant restoration in changes of paw and ankle volume and paw weight. Due to NKV treatment urinary hydroxyproline, glucosamine, serum acid phosphatase, alkaline phosphatase and IL-10 level were restored significantly, as compared with standard drug indomethacine. NKV also showed significant protection against arthritis induced oxidative damages. Thus this study confirmed the scientific validation behind ancient belief and use of snake venom in arthritis as mentioned in Ayurveda.

Ant venom (*Pseudomyrmex triplarinus*) has shown to have promising effect against rheumatoid arthritis. Antiinflammatory property of ant venom was established in carrageenin induced paw edema model[72]. In FCA induced model it also showed some beneficial effects[73]. A semi pure fraction of the ant venom possesses antiarthritic activity in arthritic patients[74].

Bee venom had been shown to have anti-arthritic activity[75]. Subcutaneous administration of bee venom suppressed the development of carragenin induced paw edema and adjuvant induced arthritis in rat model. Bee venom when administered with FCA suppresses the development of arthritis. The major component mellitin (40-50% dry wt) isolated from bee venom showed anti-inflammatory, analgesic and anti-arthritic activity. Adolapin, which was also isolated from bee venom showed analgesic and anti-inflammatory activity[76]. It also showed antiarthritic activity in other bacteria induced arthritic models[77]. The bee venom and its active components inhibited pro-inflammatory interleukins like IL-1α, TNF-α and

PLA2 activity, NO, reactive oxygen species (ROS) production[78]. It also inhibited COX2 mRNA expression in a dose dependent manner. Bee venom showed similar result in cell culture study with RAW 264.7 cell lines[79]. Clinical trials with bee venom acupuncture treatment showed improvement in arthritic patients[80]. Snake venom especially cobra venom were also tested in search of the clue for antiarthritic activity. Cobratoxin, a long chain α neurotoxin isolated from Thailand cobra venom possessed antiarthritic and analgesic activity[81]. It actually inhibited the release of proinflammatory cytokines like TNF-α or IL-1. The anti-inflammatory and anti-arthritic activity of a protein toxin (NK-CT1) from the Indian monocellate cobra venom increased anti-inflammatory cytokines and it also prevented the oxidative damages in FCA induced arthritic rat model (unpublished data).

Gomes *et al*[82] reported that the Indian black scorpion *Heterometrus bengalensis* venom possessed an anti-osteoporosis activity. It was found that this venom significantly restored urinary $Ca^{2+}$, $PO_4^{3-}$, creatinine (CRE) and hydroxylproline (OH-P). Serum $Ca^{2+}$, $PO_4^{3-}$, tartrate-resistant acid phosphatase (TRAP), IL-1, IL-6, TNF-α, parathyroid hormone level (PTH), bone $Ca^{2+}$, $PO_4^{3-}$, Mg (2+), Zn (2+) and serum alkaline phosphatase (ALP), estrogen (EST) and PTH. This study confirmed that the Indian black scorpion venom may influence bone remodeling process by stimulating bone formation and reducing bone resorption process of osteogenesis.

Halder *et al*[83] reported the presence of a high molecular weight protein (bengalin) from the scorpion (*Heterometrus bengalensis*) venom having anti-osteoporosis activity in female albino wister rat. Bengalin was purified through DEAE cellulose ion-exchange chromatography. Bengalin was found to have 72 KDa and the first 20 amino acid sequence was found to be GPLTILHINDVHAA/RFEQ/GF/GNT. Bengalin antagonized osteoporosis by restoring urinary $Ca^{2+}$, $PO_4^{3-}$, CRE and OH-P, serum $Ca^{2+}$, $PO_4^{3-}$, ALP, TRAP, PTH, $T_3$, TSH, osteocalcin, IL-1, IL-6 and TNF-α and bone mineral $Ca^{2+}$, P, $Mg^{2+}$, $Zn^{2+}$, $Na^+$. Bone mineral density of osteoporosis female rats were improved due to bengalin treatment observed through DEXA scan.

## Conclusions

This review has tried to focus on the different zoo products which are traditionally used against rheumatoid arthritis. Some of them were tested in different experimental arthritis conditions in recent years. Very few active fractions and their mechanism of action have been identified. Controversy still exists regarding the use of animals, animal products that are being used either killing or hurting the animal, which affect the biodiversity. Future research opportunities are open in this area with proper care and permissions, which may yield new drug development clues against arthritis, a major socio-medical problem of the decade.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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