

STRUCTURAL AND ULTRASTRUCTURAL EXAMINATION OF ANTI-ARTHRITIC EFFECT OF BEE VENOM IN TEMPOROMANDIBULAR JOINT WITH INDUCED RHEUMATOID ARTHRITIS IN ALBINO RATS

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DOI: 10.21608/dsu.2022.93870.1079

Manuscript ID: DSU-2109-1079

KEYWORDS

Anti-arthritis, Bee venom, Carrageenan, Inflammation, Rheumatoid arthritis, Temporomandibular joint.

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ABSTRACT

Introduction: Bee venom is broadly utilized in conventional Chinese medication for the treatment of fiery maladies, such as rheumatoid arthritis and to lighten the related pain. **Aim:** This research aimed to investigate the anti-arthritic impact of bee venom in repairing the possible structural and ultrastructural changes in the temporomandibular joint of albino rats with induce rheumatoid arthritis. **Material and Methods:** Thirty adult male albino rats were used in this investigation. The animals were divided into; Group 1: (n=10) served as negative control; Group 2: (n=20) they received 7 intra-articular injections of 0.02 ml of carrageenan solution in order to induce Rheumatoid Arthritis. Then after, the animals of group 2 were divided equally into; Subgroup 2.1: served as positive controls; Subgroup 2.2: were treated by subcutaneous injection of 0.2ml of bee venom solution for 12 weeks. At the end of the experiment the rats of different groups were euthanized then the head of each animal was divided sagittally into two halves; the right halves of all groups were used for structural examination while the left halves were used for ultrastructural examination. **Results:** Biochemical results revealed subgroup 2.2 showed a significant decrease in the values compared with subgroup 2.1. Histological and electron microscopic examination of the present sections of subgroup 2.1 showed variable microscopically degenerative changes compared to control group 1; while the present sections of subgroups 2.2 revealed marked improvement of the different components of rat's temporomandibular joint. **Conclusion:** Bee venom have antiarthritic effect in arthritic rat's temporomandibular joint.

INTRODUCTION

The temporomandibular joint (TMJ) is a diarthroidal and synovial joint at the base of the skull that connects the mandible to the temporal bone ⁽¹⁾. It's made up of the squamous section of the temporal bone and the mandible's condyle. An intervening disc of connective tissue articulates these two osseous pieces, which are contained in a fibrous capsule ⁽²⁾. As a result, the joint cavity is separated into two compartments: upper and lower. Except for the articulating surfaces, the capsule and disc have a synovial membrane on the inside. This inner membrane produces synovial fluid, which fills the joint compartments ⁽³⁾.

In clinical settings, TMJ arthritis caused by rheumatoid arthritis or osteoarthritis is frequent. The inflammatory response can harm the joints and impair their ability to function normally⁽⁴⁾.

Rheumatoid arthritis (RA) is a systemic inflammatory disease marked by symmetric polyarthritis, synovial inflammation and hyperplasia, and cartilage and bone destruction⁽⁵⁾. Although the exact cause of RA is uncertain, many experts agree that the deterioration of the joint's bone and cartilage is caused by activated inflammatory T cells, B cells, and macrophages infiltrating the synovium⁽⁶⁾. These inflammatory cells release various pro-inflammatory cytokines and mediators, which cause severe tissue damages and secondary inflammatory injuries in arthritis⁽⁷⁾. Besides, T cells also initiate the autoimmune process and mediate chronic synovitis, leading to joint destruction⁽⁸⁾.

As adjunctive therapy in the treatment of RA, symptomatic drugs that act in the control of pain and inflammation such as analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARD) and steroids (corticosteroids) are recommended⁽⁹⁾.

NSAIDs inhibit cyclooxygenase enzymes (COX-1 and COX-2) and reduce pain and inflammation by restraining the formation of prostaglandins⁽¹⁰⁾. Due to the reduction of prostaglandins production in the gastrointestinal mucosa, NSAIDs can cause gastric damage and compromise cardiovascular safety⁽¹¹⁾.

The European Alliance of Associations for Rheumatology (EULAR) recommends the use of a low-dose corticosteroid as part of the initial treatment strategy in combination with DMARD for up to 6 months, decreasing the dose as clinically as possible⁽¹²⁾.

Systematic review published in 2004 found that the use of low-dose prednisolone (maximum 15mg/d) was superior to placebo and NSAIDs in improving joint sensitivity and pain in patients with RA, but the authors reported some limitations of the study as poor description of adverse effects, substantial heterogeneity between clinical trials and re-

striction of findings only at the first month of treatment initiation⁽¹³⁾. Due to, several disadvantages of the medications, such as severe side effects and exorbitant costs, limit their use⁽¹⁴⁾. As a result, as a typical alternative medicine method, a new therapeutic strategy is required to treat RA that not only prevents joint degeneration but also has fewer side effects and lower costs.

Bee venom (BV) is a fragrant light-yellow liquid released by bees that have been aroused. Melittin, phospholipase A2, histamine, hyaluronidase, catecholamine, and serotonin are all found in this substance⁽¹⁵⁾. Melittin is the most important medicinal component, accounting for half of the dry weight of BV. Melittin has potent anti-inflammatory and analgesic properties, and it can be used in both natural and synthetic membranes. It has hormone-like properties but none of the adverse effects of hormones⁽¹⁶⁾. It can increase adrenocorticotropic hormone secretion, decrease capillary permeability, and inhibit prostaglandin E2 and neutrophil production⁽¹⁷⁾. Melittin's analgesic intensity is 40% that of morphine and its analgesic duration is twice as long. BV does not influence the digestive tract in the same way that salicylic acid-based medicines do, and it does not suppress the immune system in the same way that corticosteroids do⁽¹⁸⁾.

Studies on Bee Venom Therapy in the field of RA were published since 1970, Li *et al.*⁽¹⁹⁾ used Freund's adjuvant-induced animal model to induced arthritis and found that treatment with BV suppressed FOS expression in the superficial layer of the lumbar spinal cord and thus reduced paw edema and nociceptive behaviors in the injected side of the paw. On the other hand, in a type-II collagen-induced arthritis (CIA) model, Lee *et al.* found that BV injection therapy inhibited immune responses. TNF- α production was notably lower in the BV group than in the control group, while IL-1 β remained the same level⁽²⁰⁾. In addition, A study by Darwish *et al.*

found combination of BV and methotrexate potentiated the anti-arthritic effects of methotrexate and suppressed hepatotoxicity induced by methotrexate due to the decreased expression of TNF- α and NF- κ B (p65) in the synovial membrane of the hind paw⁽²¹⁾. These studies indicated that BV injection had antiarthritic and antinociceptive effects on rats with arthritis and can be an alternative therapy for rheumatoid arthritis.

For better understanding of human rheumatoid arthritis, experimental animal models of inflammatory arthritis are useful. Adjuvant arthritis, collagen-induced arthritis, and streptococcal cell wall arthritis are the three main models of chronic arthritis in rats now in use⁽²²⁾. Carrageenan-induced arthritis has been employed in rats, rabbits, dogs, and other animals.

Carrageenan, often known as Irish moss or carrageen moss, is a sulphated mucopolysaccharide derived from the seaweeds *Chondrus* spp. and *Gigartina* spp. It's well-known for its ability to cause local inflammation dominated by macrophage aggregation and fibroblastic proliferation⁽²³⁾.

In the present work, it was worth shedding light on the possible anti-arthritic effect of bee venom in repairing the possible structural and ultrastructural changes in the temporomandibular joint of albino rats with induced rheumatoid arthritis.

MATERIALS AND METHODS

Approval of Research Ethics Committee, Faculty of Dentistry, Suez Canal University was obtained before starting this controlled experimental study (54/2017).

This study was conducted on 30 adult male albino rats; with average weight 160-180 g. Animals were divided into the following groups:

Group 1: consisted of 10 rats and served as negative controls.

Group 2: consisted of 20 rats and served as experimental group. Over 21 days, they received 7 intra-articular injections of 0.02 ml of sterile 1% carrageenan in 0.9% saline solution (one injection every 3 days) in the right and the left temporomandibular joints under general anesthesia for the induction of Rheumatoid arthritis.

At day 24, **blood samples** were collected from the retro orbital plexus of veins at the inner canthus of rat's eyes for laboratory investigations to detect (RF, ESR and CRP) to confirm diagnosis of bilateral TMJ involvement by RA in which these investigations increase more than normal.

Then after, the animals of group 2 divided into two subgroups as follows:

Subgroup 2.1: consisted of 10 rats with rheumatoid arthritis. They served as positive controls.

Subgroup 2.2: consisted of 10 rats with rheumatoid arthritis. They were treated by subcutaneous injection with bee venom solution three times per week for 12 weeks. The initial dose was 0.2 ml and after one-week (three applications) loading dose of 0.5 ml per injection was used.

At the end of the experiment which lasted 15.5 weeks (23 days for carrageenan injection + 12 weeks for treatment by bee venom), the animals of the different groups were euthanized by cervical dislocation. Immediately after euthanization, the head of each animal was divided sagittal into right and left halves; the right halves of rat's head of all groups were fixed in 10% neutral buffered formalin, decalcified in 10% EDTA solution for two months. The specimens were then washed properly under running water, dehydrated by transferring through ascending grades of alcohol, then transferred to xylene to clear the specimens from alcohol. The specimens were

infiltrated with paraffin wax and embedded in the center of the paraffin wax blocks. The embedded specimens were sectioned 6 microns thick. The sections were mounted on clean glass slides and stained with Hematoxylin and eosin stain for histological examination and detection of any structural changes in the different components of the TMJ.

While the left halves of rat's head of all groups were fixed in 3% phosphate buffered glutaraldehyde for 4 hours, washed in the buffer for 24 hours at 4°C then they were decalcified in 10% EDTA solution PH 7-7.3. For electron microscopic examination, very small pieces (1 mm³) were immediately cut from the decalcified specimens using very sharp blade within the phosphate buffer from the corresponding sites to be examined with transmission electron microscope for ultra-structural examination of specimens after complete decalcification. Specimens washed in freshly prepared phosphate buffer PH7, post fixed in 1% buffered osmium tetroxide, washed, dehydrated in ascending grades of ethanol and then embedded in epoxy resin. Ultrathin sections (0.06 microns) were cut using ultramicrotome and glass knives and then mounted on copper grids and stained with uranyl acetate followed by lead citrate and examined with Transmission electron microscope.

Finally, examination was carried out using the T.E.M (JEOL 1000) in the cancer institute, Cairo University.

RESULTS

1. Biochemical and hematological results:

Our results showed that positive control subgroup significantly increased ($p \leq 0.05$) in the values of CRP, ESR and SRF compared with negative control group, while the bee venom treated subgroup showed a significant decrease ($P \leq 0.05$) in the values of CRP, ESR and SRF compared with positive control subgroup as in table (1).

Table (1) Statistical analysis of the values of CRP, ESR and SRF in blood samples of different groups.

Parameters	Negative control group	Positive control subgroup	Bee venom treated subgroup
CRP (mg/dl)	4.9±0.4	13.9±0.18*	5.9±0.38#
SRF (IU/ml)	50.41±2.29	100.5±1.65*	62.8±0.4#
ESR (mm/h)	3.35±0.95	10.9±0.16*	4.2±0.18#

Values were expressed as mean ± standard (n=6). (*) Significantly different from normal negative control group ($P < 0.05$). (#) Significantly different from positive control subgroup ($P < 0.05$). Student's *t* – test.

2. Histopathologic results:

Group 1 (negative control) showed normal histological architecture of the TMJ components (head of mandibular condyle, glenoid fossa of temporal bone and articular disc). The articular surfaces showed regular, smooth and intact surfaces with no undulations, fraying or flaking (**Fig. 1-A**).

Subgroup 2.1 (positive control) showed many degenerative histological changes compared to control group. These changes were variable in severity. There were increases in the thickness of the fibrous tissue layer of both mandibular condyle and glenoid fossa that might be fused with the intermediate zone of the disc. Narrowing and / or almost complete obliteration (ankylosis) of the joint space. Irregular or undulated notched articular surface of the condyle with apparent reduction in thickness of condylar cartilage. Chondrocytes appeared disorganized and few, regional loss of chondrocytes and clustering (cloning) of chondrocytes near the articular surface with disorganized matrix network and fissuring of the cartilage matrix was seen. TMJ disc showed severe fraying, tearing and cracks. The synovial membrane showed marked synovial hyperplasia (pannus) with finger like projections directed to the upper compartment of the TMJ. The intimal

lining showed marked thickening with villous formation while sub-intimal stroma showed marked increase in the collagen fiber content and apparent infiltration of inflammatory cells lymphocytes, plasma cells and macrophages (Fig. 1-B1, B2).

Subgroup 2.2 (Bee venom treated subgroup) regained most of normal joint and tissue construction and integrity when compared with subgroup 2.1. They revealed regular and smooth articular surface of both mandibular condyle and glenoid fossa and marked decrease in the thickness of fibrocartilage tissue layer of the condyle. The articular disc showed regular smooth meniscus surface with minimal cracks. Compared with the positive control specimens, there were less cartilage degenerative changes, narrowing in bone marrow spaces, less infiltrated with diffuse chronic mononuclear inflammatory cells and decreased in bone wear and resorption. Increase in the size of the upper and lower compartments was always seen (Fig. 1-C).

3. Transmission Electron Microscopic Results:

Group 1 (negative control) showed the following: Chondrocytes of the condylar cartilage were polygonal in shape with normal cytoplasm contains well-developed rough endoplasmic reticulum, numerous well shaped Golgi apparatus, mitochondria and large vesicular euchromatic nucleus, their plasma membranes bears processes called filopodia extending into an electron-lucent pericellular zone. Fibroblasts of the disc and the fibrous covering of the condyle and glenoid fossa showed fusiform shape containing a large open face nucleus and very small amount of cytoplasm rich in rough endoplasmic reticulum, rod-like shaped mitochondria, a large Golgi apparatus and short and thick processes. Osteocytes of the condyle and glenoid fossa appeared normal, entrapped into lacunae, several long processes extended inside the canaliculi. Their cytoplasm contains few normal mitochondria and very few cisternae of granular endoplasmic reticulum (Fig. 2).

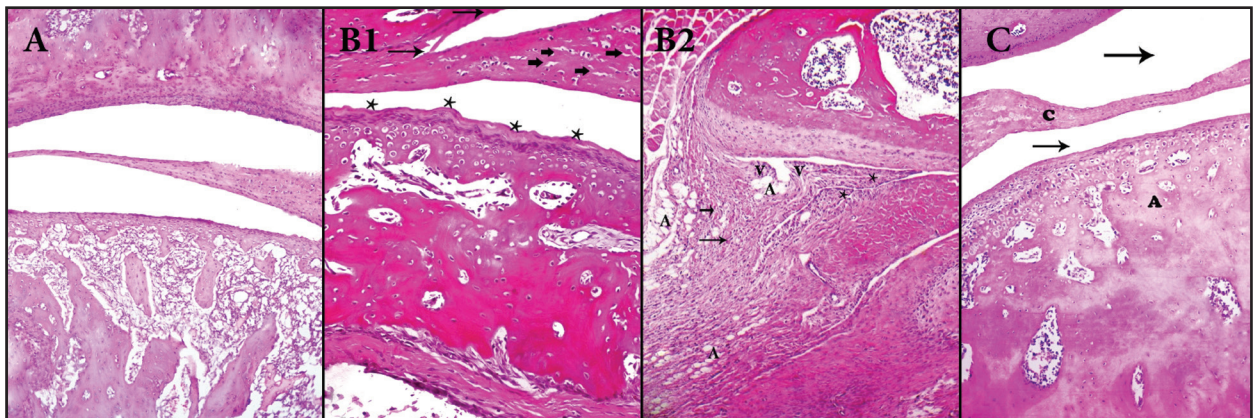


Fig. (1) A combined picture representing histopathologic sections of rat TMJ of all groups. A: group 1 showing normal histological architecture of the TMJ components (H&E orig. mag. 100). B1: subgroup 2.1 showing irregular articular surface of the condyle (*), apparent reduction in thickness of cartilage and destruction and flaking of articular surface of the glenoid fossa (thin arrows) and a lot of cracks in the disc (thick arrows) (H&E. orig. mag. 200). B2: subgroup 2.1 showing synovial hyperplasia with finger like projections entering the upper compartment (*), the intimal lining with marked thickening and villous formation (v) while sub-intimal stroma appeared with marked thickening and apparent infiltration of mononuclear inflammatory cells, proliferative and dilated congested blood vessels (arrows) and abundant lipid droplets (A) (H&E orig. mag. 200). C: subgroup 2.2 showing smooth articular surfaces of both condyle (A) and glenoid fossa (B) with decrease in the thickness of fibrocartilage tissue layer of the condyle. The upper and lower compartments are wide (arrows) and the articular disc (c) is regular and smooth with decrease in its thickness especially in intermediate band and decrease in its cellularity and vascularity (H&E. orig. mag. 100).

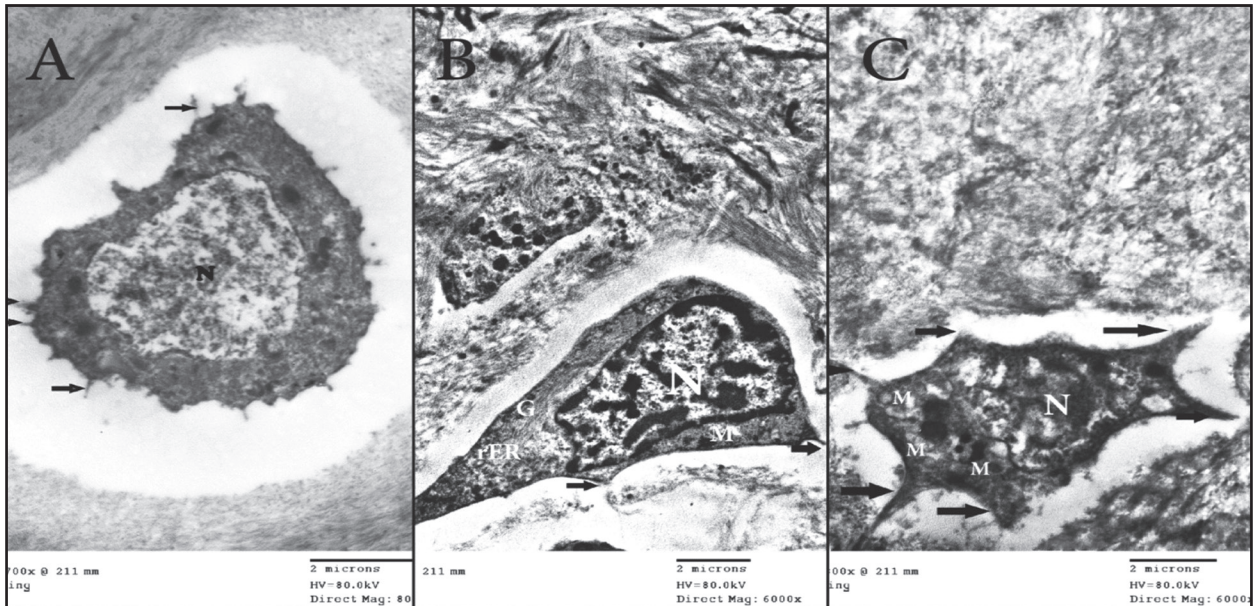


Fig. (2) Transmission electron micrograph of group 1 showing A: chondrocyte of the condylar cartilage is polygonal in shape with normal cytoplasm, contains large vesicular nucleus (N). Their plasma membrane bears some relatively long filopodia (arrows) (orig. mag. 8000). B: disc fibroblast with large open-faced nucleus (N) and small amount of cytoplasm rich in rough endoplasmic reticulum (rER), rod like shaped mitochondria and a large Golgi apparatus (G) (orig. mag. 6000). C: osteocyte entrapped into lacunae within decalcified bone matrix, several long processes extends inside the canaliculi (arrows). The cytoplasm contains large nucleus (N) and few normal mitochondria (M) (orig. mag. 6000).

Subgroup 2.1 (positive control) showed the following: Necrotic and degenerated chondrocytes represented by irregular and empty lacunae were frequently seen. Degenerated fibroblast from articular zone of mandibular condyle had a fusiform shape and the superficial collagen fibrils are disrupted. They were poorly adapted to the surrounding matrix and contained degenerating mitochondria, lysosomes, dark electron-dense vacuoles, cell-derived lipid debris, their cell processes were retracted and their nuclei contained electron-dense clumps of chromatin. Osteocytes of the condyle and glynoid fossa showed a flattened appearance. Some mitochondria were swollen, disrupted and they demonstrated cytoplasmic vacuolization and few cell processes. Multinucleated osteoclasts in their Howship's lacuna with prominent cytoplasm having a lot of mitochondria and the characteristic ruffled border were frequently seen (**Fig. 3**).

Subgroup 2.2 (Bee venom treated subgroup) there was marked improvement in the ultrastructure of the different components of the TMJ as follows: Well preserved chondrocytes of the condylar cartilage. They were regular polygonal in shape and centrally located in their lacunae, had large vesicular nuclei surrounded by faint cytoplasm with few organelles, relative long and numerous filopodia, collagenous matrix appeared intact. Fibroblasts of the disc and the fibrous covering the condyle and glynoid fossa appeared normal fusiform in shape with large piriform cell body and well adapted to surrounding matrix; They had large open face nucleus and rod like mitochondria. Osteocyte of the condyle and glynoid fossa appeared normal, entrapped into lacunae, several long processes extended inside the canaliculi. Their cytoplasm contains normal mitochondria and few apoptotic bodies (**Fig. 4**).

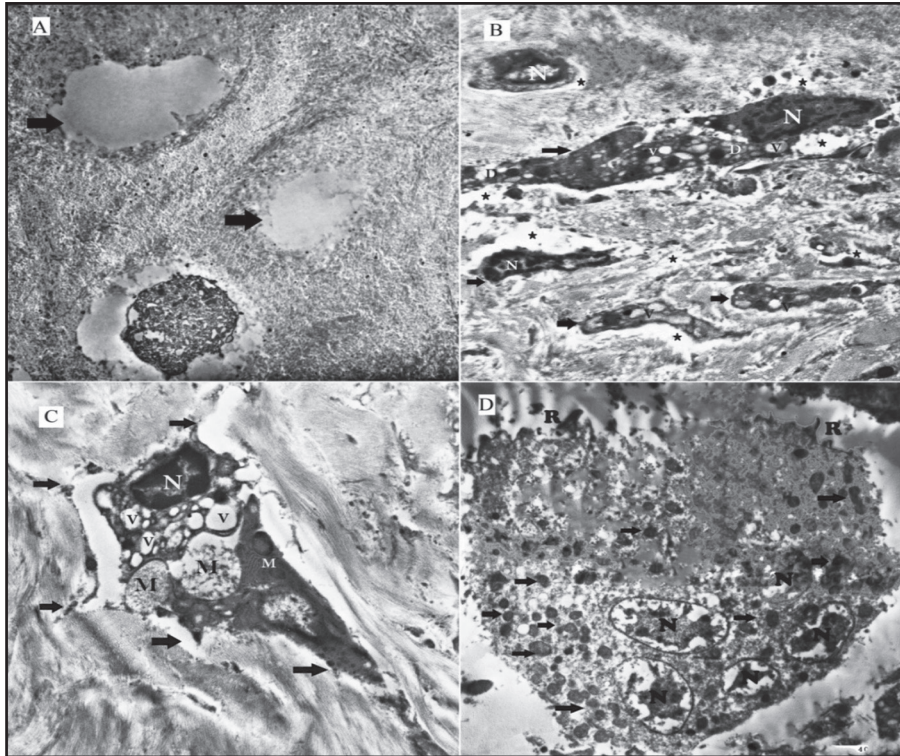


Fig. (3)

Transmission electron micrograph of subgroup 2.1 showing A: a shrunken chondrocyte with dark nucleus and irregular and empty lacunae (arrows) (orig. mag. 3000). B: Degenerated and shrunken fibroblast from articular zone of mandibular condyle, has a fusiform shape (arrows), cytoplasmic vacuoles (V) and cell-derived lipid debris (D). Cell processes are retracted. The nuclei contain electron-dense clumps of chromatin (N) (orig. mag. 5000). C: flattened osteocyte within its lacuna. Some mitochondria (M) are swollen and disrupted and cytoplasmic vacuolization (V). Few cell processes inside the canaliculi (arrows) (orig. mag. 5000). D: multinucleated (N) osteoclast cell in its Howship's lacuna with prominent cytoplasm having a lot of mitochondria (arrows) and the characteristic ruffled border (R) (orig. mag. 4000).

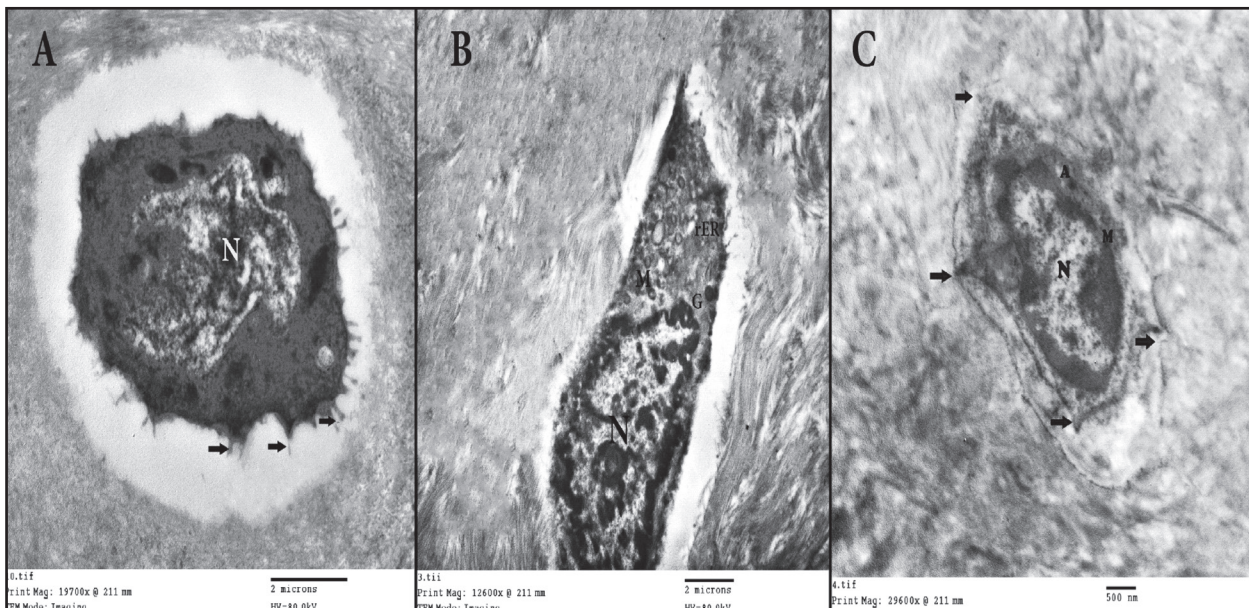


Fig. (4) Transmission electron micrograph of subgroup 2.2 showing A: chondrocyte of condylar cartilage regular polygonal in shape, centrally located in its lacunae had large vesicular nuclei (N) surrounded by cytoplasm with few organelles, filopodia (arrows). Collagenous matrix appeared intact (orig. mag. 8000). B: fibroblast of the disc had large piriform cell body containing a large open face nucleus (N) and very small amount of cytoplasm rich in rough endoplasmic reticulum (rER), rod-like shaped mitochondria (M), a large Golgi apparatus (G) (orig. mag. 6000). C: Osteocyte appeared normal shape within its lacuna, several long processes extended inside the canaliculi (arrows). The cytoplasm contains nuclei (N), normal mitochondria (M) and few apoptotic bodies (A) (orig. mag. 12000).

DISCUSSION

Albino rats were used as experimental models in this study because their TMJ is anatomically and structurally similar to human TMJ, and they share many pathologic characteristics with RA in humans, such as swelling of the extremities, synovium hyperplasia, and cartilage degradation, making it possible to test new drugs for the treatment of RA⁽²⁴⁾. Only male rats were bred for this study to reduce the interference of estrogen, which is known to have a tremendous effect on bone and cartilage health in itself⁽²⁵⁾.

In our study, the treatment by subcutaneous injection of 0.2 ml of BV solution lasted for 12 weeks this agree with previous study that conducted on 538 participants received weekly dermal injections of BV or histamine. After 12 weeks, the BV biotherapy group showed a remarkable improvement over the control group in Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain scores⁽²⁶⁾.

In this study, arthritis was induced in rats by injecting carrageenan intra-articularly into their temporomandibular joints. Carrageenan was chosen because it can elicit a highly repeatable local antigenic inflammatory response⁽²⁷⁾.

Carrageenan has been demonstrated to stimulate inflammatory cells to release lysosomal enzymes, specifically p glucuronidase. These enzymes are involved in articular injury in a big way⁽²⁸⁾. Oliveira *et al.* demonstrated that injecting carrageenan into the joint stimulates neutrophils to infiltrate the joint. The neutrophils are triggered to release substances when they come into touch with articular cartilage⁽²⁹⁾.

In the present study the diagnostic biochemical tests examination of the blood samples of the positive control subgroup 2.1 revealed highly signifi-

cantly increase in the levels of ESR, CRP and RF, when compared with the negative control group; these findings agree with previous study conducted by Rachchh and Galani⁽³⁰⁾.

In the current study examination of H&E-stained sections of positive control subgroup 2.1 when compared to the negative control group, the intra-articular carrageenan injection caused various microscopically degenerative alterations. The erosion of the condylar fibrous and cartilaginous layer, significant degenerative alterations in the TMJ. There is resorption of the cartilaginous zone and the cartilage-bone interface, as well as resorption of the condyle's surface with widening of marrow spaces and necrosis of marrow tissue, and fusion of the articular disc's central part to the fibrous and cartilaginous layers of the mandibular condyle. The chondrocytes of the condylar cartilage were atrophied, as shown by their varied size decrease and pyknotic nuclei. Hyperplasia (punnus development) and significant proliferation of synovial villi into the temporodiscal region, as well as infiltration of chronic inflammatory cells, were also seen in the synovial membrane.

These findings were in the same line and agree with those revealed by many previous investigators Kilic *et al.* who proved that TMJ-RA produces focal joint cartilage degeneration due to decreased chondrogenesis, which is accompanied by increased chondrocyte death in the growth plate cartilage, erosion, flattening, and the production of osteophytes⁽³¹⁾.

Also, Huebner *et al.* revealed the same observations as a result of mechanical osteoarthritis induction, the synovial membrane is damaged⁽³²⁾. Braun *et al.* showed that intense inflammatory infiltration, synovial membrane hyperplasia with villous alterations, and thickening of the condylar articular surface with fibrocartilage atrophy⁽³³⁾.

Xu *et al.* also found that the arthritic groups demonstrated features associated with our findings include a steady decline in chondrocyte number and condylar cartilage thickness over time, expanded bone marrow cavities, and disorganized trabecular bone structures. They attributed their findings to a higher ratio of receptor activator of nuclear factor B ligand (RANKL) to osteoprotegerin (OPG) in subchondral bone, implying enhanced osteoclastic activity and bone loss⁽³⁴⁾. Sanchez *et al.* also stated that OPG deficiency caused cartilage thickness to decrease and chondrocyte death to increase⁽³⁵⁾.

These investigations were compatible with our study, and it also come in agreement with the present ultrastructural results of positive control subgroup 2.1 which revealed typical apoptotic features, including cell shrinkage, nuclear condensation, vacuolar degeneration, and apoptotic bodies. Degenerative changes were detected in the chondrocytes, fibroblasts and osteocytes including irregular contours, atrophied cell bodies, scanty cytoplasm, loss of cell processes, and dark irregular nuclei or vacuolated cytoplasm with many empty lacunae. These findings were like those revealed by Abdel-azeem *et al.*⁽³⁶⁾

The results of the present study showed that the subcutaneous injection of bee venom solution (0.2 ml) three times per week for 12 weeks exhibited marked potent anti-arthritic activity based on biochemical examination which was found to significantly decrease in ESR, RF and CRP values compared with the positive control subgroup 2.1. These findings agree with previous studies examining the use of methotrexate and BV⁽³⁷⁾.

Examination of the present H&E-stained sections of bee venom treated subgroup 2.2 showed regular and smooth articular surfaces of both mandibular condyle and glenoid fossa and marked decrease in the thickness of fibrocartilage tissue layer of the condyle. The articular disc showed regular

smooth surface with minimal cracks, small count of inflammatory cell infiltration, mild synovitis, no pannus formation and no apparent damage to the cartilage or the bones, when compared with the positive control subgroup 2.1.

These results were like those revealed by Kang *et al.* who proved that BV treatment preserved the joint by reducing articular cartilage loss and inflammatory cell infiltration, as well as preventing the development of leukocytosis⁽³⁸⁾.

These structural finding also come in agreement with the present ultrastructural results of subgroup 2.2 which revealed marked improvement of the different components of the TMJ as follows: well preserved chondrocytes, fibroblasts and osteocytes were detected with regular contours, large cell bodies, long and numerous cell processes, and regular large vesicular nuclei surrounded by faint cytoplasm with few organelles and few apoptotic bodies.

The antiarthritic effects of BV could be attributed to its inhibitory effects on the production of inflammation mediators such as prostaglandin E2 (PGE2), nitric oxide (NO), and tumor necrosis factor alpha (TNF), as well as the release of reactive oxygen species (ROS) that cause oxidative damage, cyclo-oxygenase-2, phospholipase A2, and intracellular calcium⁽³⁹⁾. The inflammatory pathway is blocked as a result of all these inhibitory actions.

From the aforementioned structural, ultrastructural, results it is obvious that marked arthritic and degenerative changes of the different components of rat's TMJ that occurred after the last seventh intra-articular injection of carrageenan. Using complementary and alternative medicines in nature as bee venom had marked decreased the extent of these degenerative changes and enhanced the integrity and regeneration of the rat's TMJ tissues based on its immunomodulatory, anti-arthritic, anti-inflammatory and anti-apoptotic activities.

CONCLUSION

The present study supported that the use of complementary and alternative medicines as Bee venom produced ameliorative effects on carrageenan induced rheumatoid arthritis in albino rats as they successfully revert the TMJs into a nearly normal condition via its anti-inflammatory and immunomodulatory potentials.

RECOMMENDATIONS

This study could be an initial step that needs to be tested further in humans before complementary medicines could be considered a routine and effective treatment for human rheumatoid arthritis, hoping besides apparent efficacy; their adverse effects to be relatively benign.

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