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Protective Effect of *Boswellia carterii* and Gum Arabic *(Senegalia senegal*) on Induced Arthritic in Male Rats

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RESEARCH ARTICLE

Protective Effect of *Boswellia carterii* and Gum Arabic (*Senegalia senegal*) on Induced Arthritic in Male Rats

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ABSTRACT

The study aims to use natural substances like Senegalia senegal and Boswellia carterii to treat rheumatoid arthritis, a painful disease affecting joints and making movement difficult, , The two plants were used to treat the symptoms of induced arthritis in male rats by using the complete freund's adjuvant method. A group of thirty adult male rats was used in this study, divided into six groups, with five rats for each group. The 1st group was the negative control (without arthritis induced and without treatment); the 2nd group is the positive control group (with arthritis induced and without any treatment); the third group (arthritis induced and treated with 250 mg/kg B.W *Boswellia carterii*); the fourth group (arthritis induced and treated with400 mg/kg B.W. *Senegalia senegal*); the fifth group (arthritis induced and treated with *Boswellia carterii* and *Senegalia senegal*) and the 6th arthritis induced and treated with 0.75 mg/kg of drug methotrexate, The treated and affected joints underwent histological studies after six weeks of experiments, revealing significant changes in the affected joint tissue. The study found that arthritic animals intubated with Senegalia senegal showed normal joint cavity and epiphyseal ends. while those intubated with Boswellia carterii showed thinning of articular cartilage and synovial membrane and clear chondrocytes. Results of *Senegalia senegal* and *Boswellia carterii* showed thinning of articular cartilaginous and normal cavity with normal epiphyseal ends and hyperplasia of fibroblasts in the synovial membrane. In animals receiving methotrexate, they showed normal articular cartilaginous surfaces, narrowing joint cavities, and synovitis.

Keywords: Arthritis, *Boswellia*, Knee joint, Gum Arabic, Methotrexate

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder.[1] It is characterized by leukocyte infiltration, synovial cell hyperplasia, and an invasive inflammatory pannus that is an abnormal extra layer of tissue in joints that causes pain, swelling and damage in cartilage and bones, as well as it causes damage to adjacent cartilage and bone.[2] The majority of kinds of arthritis have an unpredictable path of exacerbations and remissions, leading to varied degrees of physical deformity. Arthritis is one of the most prevalent chronic illness problems, impacting millions of individuals worldwide. Treatments aim to minimize pain and inflammation, and address inflammation-related symptoms while maintaining or improving function.[3]

The synovium is the primary target of the limiting, advanced, chronic multisystem disease rheumatoid arthritis (RA), which additionally damages the articular cartilage and erodes the juxta-articular bone. The disease has a progressive joint deformity and damage history, and a sizable proportion of patients also acquire extra-articular symptoms. Successful

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continuous therapy approaches, especially if the condition is identified and treated early, result in significant clinical benefit for the majority of patients.[4]

In this study we used a natural substance that can function as a biologically active substance against chronic inflammatory disease. These natural substances include *Senegalia senegal* (gum Arabic) and *Boswellia carterii*. *Boswellia carterii* is aromatic oleogum –resin secreted from *Boswellia carterii*, reputed as Frankincense. It is widely used in traditional medicine. *Boswellia carterii* is belonging to the family of *Burseraceae*.[5] It exudes the resin of the *Boswellia carterii* species, which is cut to allow a white, milky resin to flow[6] Frankincense, or olibanum resin, comes from the tree of the genus *Boswellia carterii,* species Boswellia (family Burseraceae).[7] It has anti-arthritic and anti-inflammatory properties.[8,9] Studies showed that the effect of Boswellic acid in Bovine serum albumin (BSA) causes arthritis in rabbits.[10,11] In addition, *Boswellia* has antioxidant activity, as the oxidative damage causes human aging, and *Boswellia carterii* eliminates free radicals[12] and inhibits lipid peroxide (Malondialgehyde)(MDA).[13]

Gum Arabic (GA) is a glutinous or gummy exudation from the trunks of acacia species, Acacia Senegal.[14–16] GA is a natural compound that was shipped from Arabian ports.[17] It is the other natural substance that was used in this study, which has anti-inflammatory properties by decreasing inflammatory markers and disease severity scores among rheumatoid arthritis and antioxidant activity via the increasing biosynthesis of antioxidant biomolecules.[18,19] Methotrexate is a drug that was used in the treatment of rheumatoid arthritis that treats inflammatory arthritis.[20] That drug has side effects that cause congenital anomalies with impaired renal function and cause bone marrow suppression and hepatotoxicity.[21] It acts through inhibition of purine and pyrimidine synthesis and reduction in T-cell dependent proliferation and suppression of inflammation.[22]

Materials and methods

Experimental animals

Thirty adult male Sprague–Dawley albino rats weighing 20–300g at the age of 10–12 weeks were purchased from the animal house. They were kept for two weeks in special plastic cages with wood shavings to raise rats with metal caps so as to allow for adaptation before treatment under the controlled temperature condition of 25 ˚C. Animals were provided with rat pellets and tap water for feeding and drinking.

Drugs and chemicals

Methotrexate drug was purchased from (Health Step Pharmaceutical) at 50 mg/5 ml. Complete Freund's adjuvants (CFA) from Santa Cruz Biotechnology. Stains used for histology include hematoxylin and eosin stain and decalcification by formic acid.

Induction of Arthritis

Rheumatoid arthritis was induced in rats by using a complete Freund adjuvant according to[23] Arthritis was induced by injecting 0.1 ml of complete Freund adjuvant (CFA) in the right foot of the rat and measuring foot thickness by machine Caplier vernier before the arthritis induction and after 24 hours of injection. Symptoms such as redness, severe swelling, stiffness, and increased thickness of the foot arthritis develop within 10–45 days after injection.[24] Then at the end of the experiment, a knee joint for the right and left sides was taken for the histological study for all groups.

Experimental design

Thirty adult male rats were used in this experiment and divided randomly into six groups. The animals weight was 200–250g each group consisted of five rats as follows: Group 0: negative control. Group 1: positive control (arthritis induced, untreated). Group 2 : *Senegalia senegal* (gum Arabic), 400 mg/kg B.W.[25] Group3: *Boswellia carterii,* 250 mg/kg B.W.[26] Group4 : *Senegalia sengal* (gum arabic) and *Boswellia carterii* Group(5): intraperitoneal (i.p.) 0.75mg /kg B.W. drug methotrexate.[27]

Preparation of aqueous extract

Boswellia carterii and *Senegalia senegal* were obtained from a herbal shop in Baghdad Governorate. They were cleaned of impurities and ground with a grinder to obtain a very fine powder. We prepared a watery decoction of *Boswellia carterii* and *Senegalia senegal*. *Senegalia senegal* (gum Arabic) 400 mg / kg B.W[26] and *Boswellia carterii* 250 mg/kg B.W.[26] Then, the extracts were stored in a clean glass container in the refrigerator and extracts were stored at 4˚C in a glass container until use.

Histology and decalcification of sample

Arthritic animals and healthy animals treated all groups G0, G1, G2, G3, G4, and G5 were scarified for histological study for knee joint decalcification to

facilitate bone tissue sectioning for histology technique is routinely used for bone samples as the removal of calcium deposits simplifies the embedding procedure and cutting De La Grandmaison.[28]

Assessment of histopathological changes

Paraffin slices were prepared according to method[29] in: Dehydration, Clearing, Infiltration, Embedding, Sectioning and Staining by hematoxylin-eosin stain.

Microphotography

Histological sections were photographed using a MEIJI light microscope with a high-resolution Canon camera.

Results and discussion

The current study was carried out to determine the histological characteristics of the joint in normal and arthritic animals. That explains the inflammatory changes include degeneration with necrosis, synovitis, infiltration of the mononuclear cells MNCs, edema, congestion, and hyperplasia in all experimental groups, and in the following histological sections.

The histopathological figures of the group negative control (G0) of the joint illustrated in Figs. 1 to 4 revealed a normal appearance of the cartilaginous articular surface, joint cavity, epiphyseal cancellous bone and synovial membrane.

The histopathological figures of the group positive control (G1) showed severe destructive arthritis with associated marked damage of the articular surface, necrosis with complete damage of the opposite articular surface, detached metaphyseal cancellous bone and marked synovitis, Figs. 5 to 8.

Group G2 arthritic animals treated with Senegal (gum Arabic) intubated orally with a section of the joint show mild thinning of the articular surface with normal epiphysial ends and normal synovial membrane, Figs. 9 and 10 That improvement in histopathological changes in animals intubated with *Senegalia senegal* (Gum Arabic) was[18] due to its anti-inflammatory benefits and also due to increasing antioxidant biomolecules.[20]

The histopathological figures of the group treated with *Boswellia carterii* (G3) showed mild thinning of the articular cartilage with a slightly irregular outline, Fig. 11, while Fig. 12 showed thinning of the articular cartilage with normal joints and normal epiphyseal cancellous bone, the improvement of the joint in G3 that was intubated with Boswellia that with normalization of the joint was due to anti-arthritic and anti-inflammatory activity[14–16] that was agreed with[10] who used Boswellia for treatment of arthritis in bovine serum albumin in arthritic animals also *Boswellia carterii* has antioxidant activity due to scanning free radicals[11] and inhibiting lipid peroxidation (MAD).[13]

The histopathological figures of arthritic animals treated with *Senegalia senegal* (gum Arabic) and *Boswellia carterii* (G4) showed thinning of the articular cartilaginous surface with a smooth outline, a normal joint cavity without cellular debris, normal epiphyseal ends of cancellous bone and mild hyperplasia of fibroblasts of the synovial membrane, Fig. 13. The group G4 that was intubated with Gum Arabic and *Boswellia carterii* the normal joint cavity without cellular debris due to anti-inflammatory and antioxidant activity of both Gum Arabic and boswellia the using of these natural substances in arthritis treatment its less toxic and side effect on the liver and kidney and body function than that is caused by using the drug methotrexate[19,20] and the methotrexate can cause bone marrow suppression so the conclusion of the study that the using of natural substances that include *Senegalia senegal* (gum Arabic) and *Boswellia carterii* improve joint histopathological change with no harmful and toxic effect on liver and kidney function that drugs used for treatment of arthritis caused side effect mostly in liver and kidney function.

The histopathological Figures of arthritic animals treated with methotrexate (G5) of the joint showed a normal cartilaginous plate of the articular surface with a smooth outline, narrowing of the joint cavity, and marked thickening of the synovial membrane associated with synovitis, dilatation and congestion of blood vessels, and infiltration of mononuclear leukocytes, Figs. 14 to 16.

In this study, both clinical and histological assessments were conducted on G1, G2, G3, and G4 arthritic animals treated with *Senegalia senegal, Boswellia carterii*, and (*Boswellia carterii* + *Senegalia senegal*) and compared with the anti-inflammatory drug used for the treatment of arthritis methotrexate in G5. After the induction of arthritis, many clinical and histological features were observed that reflect the pathological changes associated with the initiation and progression of the disease.

Current understanding of arthritis indicates that inflammation associated with rheumatoid arthritis results from enhanced blood flow to the affected region due to vasodilation. This causes the capillaries to become more permeable, allowing fluid, large molecules, and white blood cells to pass through and leave the blood and enter the tissue. White blood

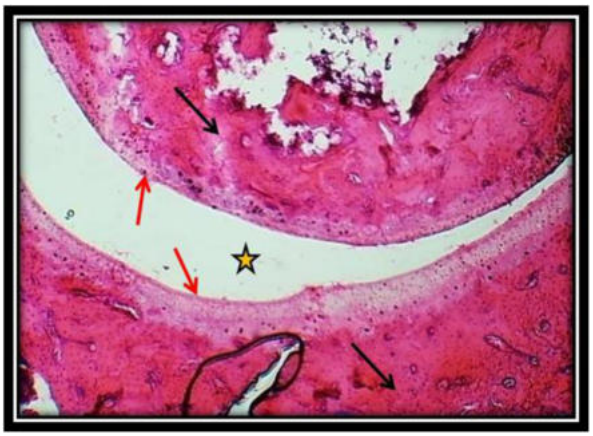

Fig. 1. Section of joint control negative (G0) shows: normal articular surface (red arrow), joint cavity (asterisk) and normal epiphyseal ends (black arrows). H&E stain. 40x.

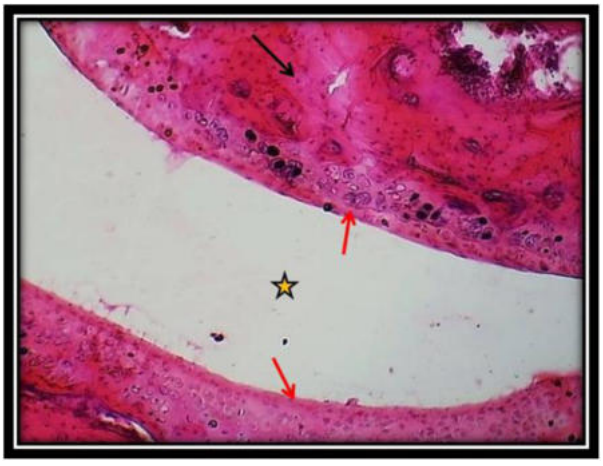

Fig. 2. Section of joint control negative (G0) shows: normal articular surface (red arrow), joint cavity (asterisk) and normal epiphyseal ends (black arrows). H&E stain.100x

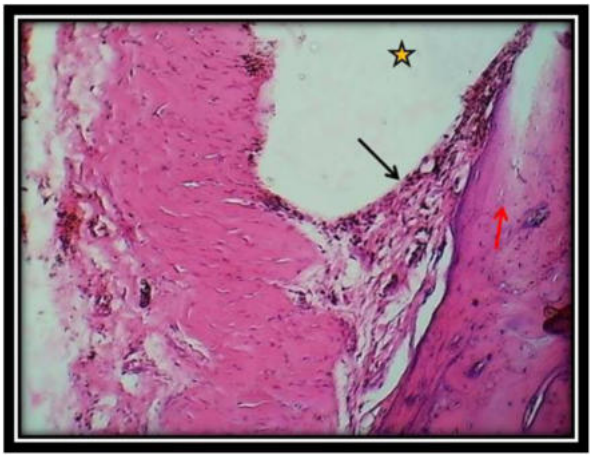

Fig. 3. Section of joint control negative(G0) shows: normal synovial membrane (black arrow), joint cavity (asterisk) and normal epiphyseal ends (red arrow). H&E stain.100x.

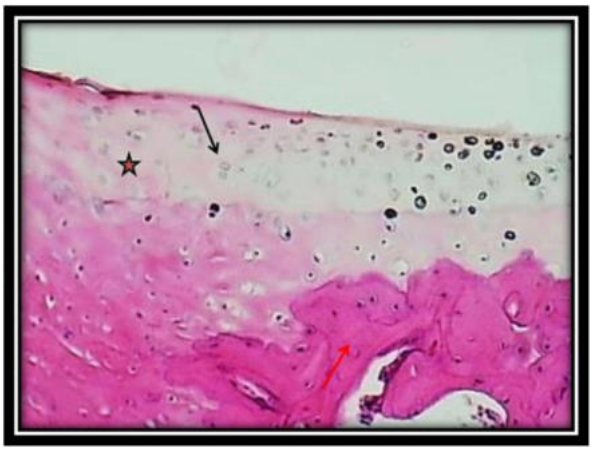

Fig. 4. Section of articular surface –hyaline cartilage control negative (G0) shows: normal chondrocyte within lacunae (black arrow), intercellular matrix is visible (asterisk) and normal epiphyseal ends of spongy bone (red arrow). H&E stain.100x

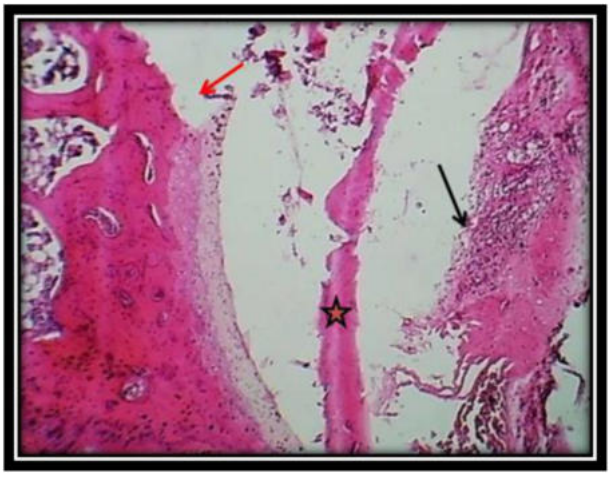

Fig. 5. . Section of joint control positive (G1) shows sever destructive arthritis with marked damage to the articular surface (red arrow), necrosis with severe damage of opposite articular surface and detached metaphyseal cancellous bone (asterisk) and synovitis (black arrow). H&E stain.40x

cells, especially neutrophils and monocytes, move to the injured site by chemotaxis. The inflammatory process is accompanied by redness, heat, swelling, and pain; redness and heat are brought on by the increased blood flow, and swelling is the outcome of the increased movement of fluid and white blood cells into the area of inflammation.[30] The current study indicated a thickening of the synovial membrane resulting from hypertrophy in membrane cells due to the action of inflammatory cytokines released from the cell's infiltration into the synovial membrane and infiltration of inflammatory cells is the result of the immune response. The very high levels were caused by the complete freund's adjuvant

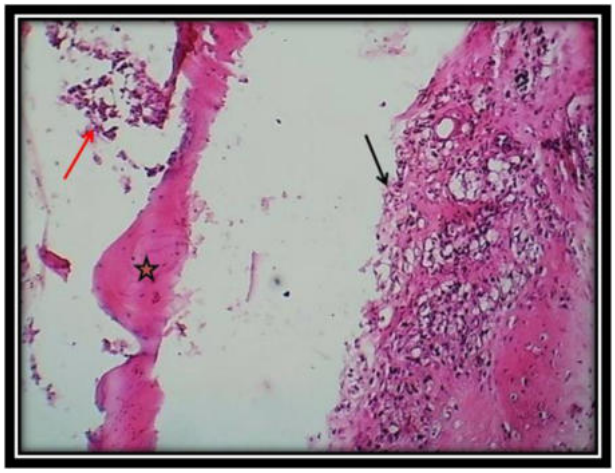

Fig. 6. Section of joint control positive (G1) shows marked cellular necrotic tissue within the joint cavity associated with necrosis damage to the articular surface (red arrow), bone detachment and synovitis of the metaphyseal cancellous bone (asterisk) & synovitis characterized by thickening of the membrane and degeneration with infiltration of MNCs (black arrow). H&E stain.40x.

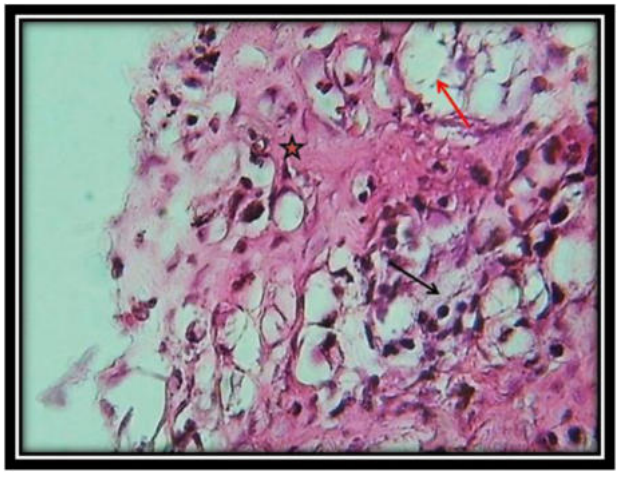

Fig. 7. Section of synovial membrane control positive (G1) shows marked degeneration with necrosis of synovial cellular content (red arrow) with necrosis of fibrous tissue (asterisk) and infiltration of MNCs (black arrow). H&E stain.400x.

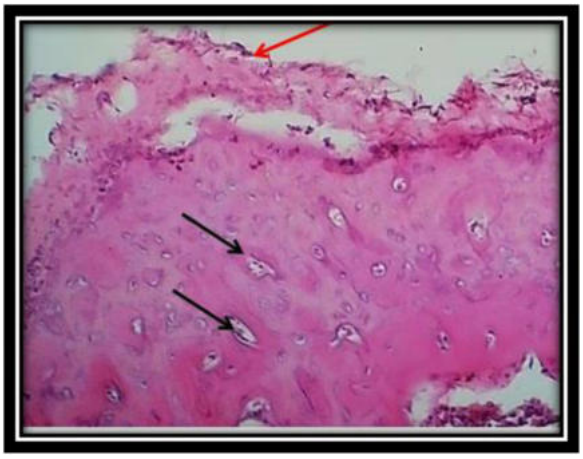

Fig. 8. Section of articular surface of hyaline cartilage control positive (G1) shows marked necrotic cellular debris covered with irregular outline of articular surface (red arrow) and necrosis with atrophy of most chondrocytes within lacuna (black arrows). H&E stain.400x.

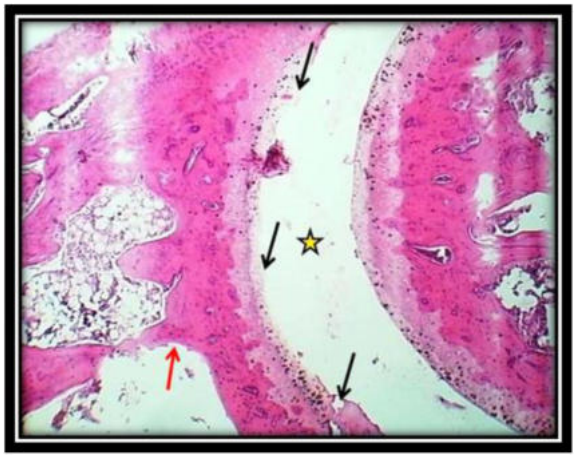

Fig. 9. Section of joint in arthritic animals intubated with *Senegalia senegal* (gum Arabic) (G2) in arthritis shows clearly articular surface thinning (black arrows), with irregular outline (black arrows), normal joint cavity (asterisk) and normal epiphyseal ends (red arrow). H&E stain.40x

(CFA) used for induction of arthritis at the site of infection, and that happened in the positive control group.[31] Histological examination showed necrosis and degeneration of chondrocytes, and that agrees with the[32] infiltration of macrophages and neutrophil cells into synovial tissue and synovial fluid, as these cells produce large quantities of free radicals[33] Activated inflammation Fibroblast-like synovial cells are activated by inflammatory mediators, such as interleukin (IL-l) and tumor necrosis factor (TNF), which are released from inflammatory cells in response to different stimuli. This inflammatory process produces active oxygen species and proteases, which lead to the breakdown of cartilage. In addition to these cells, inflammatory cytokines play a role in synovial hyperplasia and the destruction of cartilage and bone.[34,35] The necrosis of cartilage cells may result from lysosomal enzymes that play an important role in the pathology of joint tissues in arthritis; in addition, prostaglandins, leukotrienes, and cytokines (IL-1, IL-6, and TNFα) produced by autophagic cells also participate in the breakdown of joint tissue[36,37] *Senegalia senegal* and *Boswellia carterii* have demonstrated anti-inflammatory activity when used to treat arthritic animals in stages G2, G3, and G4. In this study we used some natural substances to treat arthritis because some of the drugs are

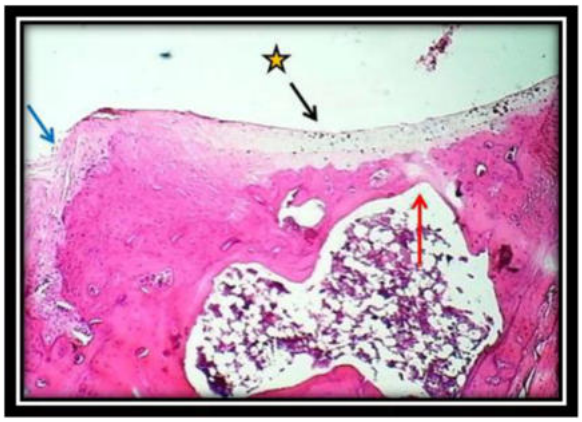

Fig. 10. Section of joint in arthritic animals intubated with *Senegalia senegal* gum Arabic (G2) shows normal articular surface (black arrows), a normal joint cavity (asterisk), and normal epiphyseal ends (red arrow). & normal synovial membrane (blue arrow). H&E stain.100x.

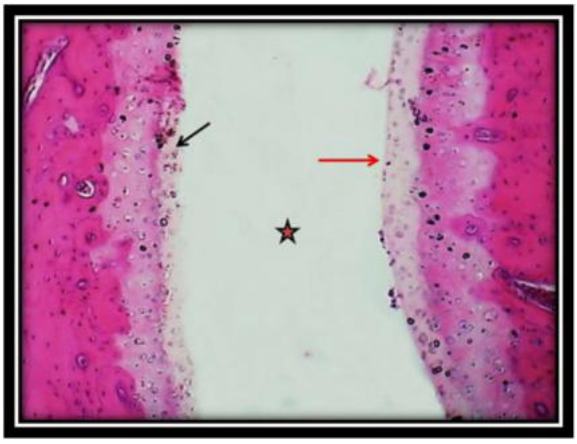

Fig. 11. Section of joint in arthritic animals intubated with *Boswellia carterii* (G3) shows thinning of articular cartilage with slightly irregular outline of the articular surface with normal chondrocytes (black arrow), normal joint cavity (asterisk) and normal opposite articular surface with smooth outline (red arrow). H&E stain.400x.

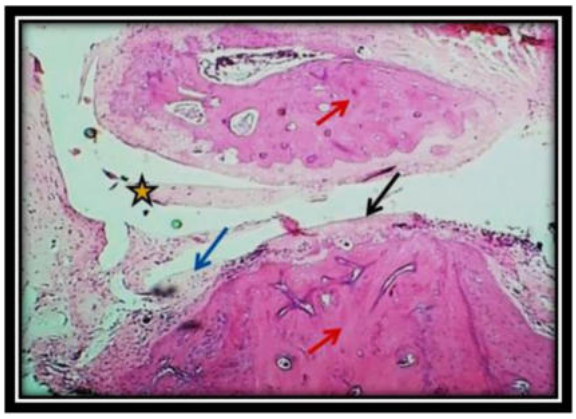

Fig. 12. Section of joint *Boswellia carterii* (G3) shows thinning of the articular surface (black arrow), normal joint cavity (asterisk), normal epiphyseal cancellous bone (red arrow) and clearly hyperplasia of fibroblasts in the synovial membrane (blue arrow) H&E stain.40x

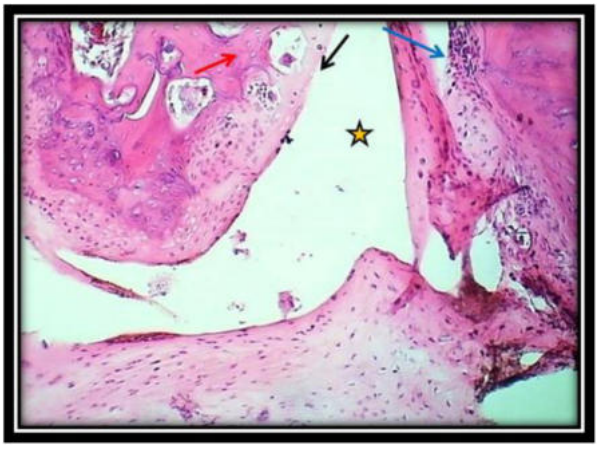

Fig. 13. Section of joint *Boswellia carterii* + *Senegalia senegal* (gum Arabic) (G4) shows dual treatment effects on joint surface synovial membrane thinning cartilaginous surface (black arrow), normal joint cavity (asterisk), normal epiphyseal ends (red arrow) and hyperplasia of fibroblasts synovial membrane (blue arrow) H&E stain.100x.

steroids and non-steroidal anti-inflammatory drugs and analgesic properties (NSAIDs) have side effects associated with the gastrointestinal tract, like gastric ulcer and some elevated blood pressure and toxic effect on the liver and kidney.[38] The use of natural substances like *Boswellia carterii* in our study elucidated the presence of a normal joint cavity and intact epiphyseal cancellous bone. Our results agree with[39] who demonstrated that *Boswellia carterii* has reduced arthritis in rats. They also agreed with[40] who reported that *Boswellia carterii* decreased knee pain in osteoarthritis patients and allowed them to walk. On the other hand, in our study, gum Arabic shows a normal articular surface, normal joint cavity, normal epiphyseal ends and normal synovial membrane. Our results agree with[41] that decreased Malondialdehyde (MAD) and Superoxide dismutase (SOD) along with elevated Glutathione (GSH) indicate that gum Arabic had antioxidant activity against free radicals, which is an important factor in any inflammatory process like arthritis. Methotrexate, in our study, explained normal articular cartilaginous surface with a smooth outline and narrowing joint cavity and little thickening of the synovial membrane with infiltration of mononuclear leukocytes. Our results agree with[42] that MTX improves pain-related behavior. It prevents the expression in the knee joint. Nonetheless, a study showed that it harmed liver and kidney tissue.[27]

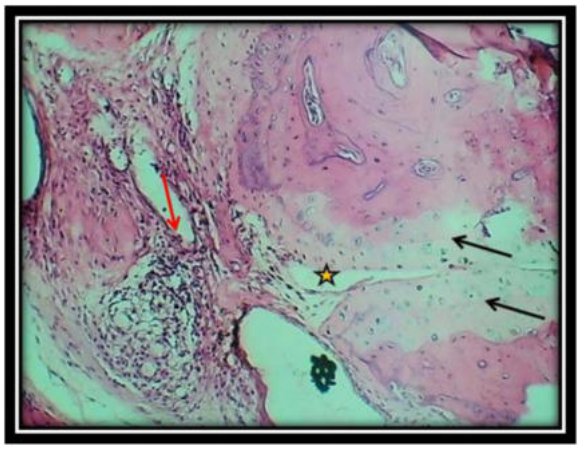

Fig. 14. Section of joint methotrexate (G5) shows a normal articular cartilaginous surface with a smooth outline (black arrow), with narrowing joint cavity (asterisk), and thickening of synovial membrane with infiltration of MNLs (red arrow). H&E stain.100x.

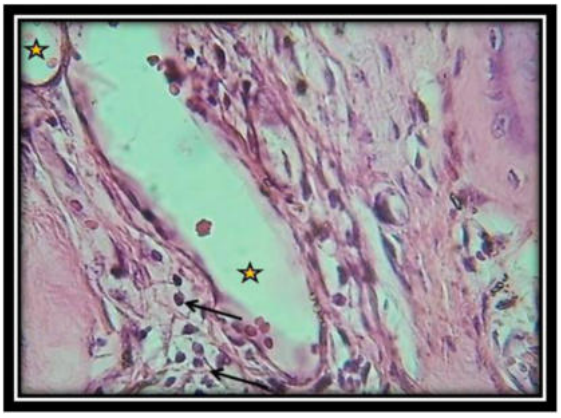

Fig. 15. Section of joint methotrexate (G5) shows synovitis characterized by infiltration of mononuclear leukocytes (black arrows) and blood vessel dilation and leukocyte infiltration with distinct arrows (asterisks). H&E stain.400x.

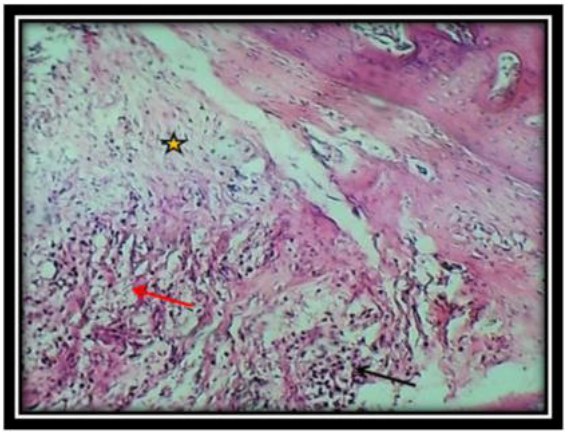

Fig. 16. Section of joint methotrexate (G5) shows synovitis characterized by thickening of synovial membrane with edema (red arrow) & infiltration of mononuclear leukocytes (black arrow). H&E stain.100x.

Conclusion

The induction of arthritis in male rats and comparing between using natural substances that include *Boswellia carterii* and *Senegalia sengal* (Gum Arabic) had a better therapeutic effect on inflammatory changes than the drug Methotraxate, as explained in histopathological change. Other studies are required to compare the side effects produced by the drug methotraxate, which was mildly used for rheumatoid arthritis treatment on liver and kidney function tests. Also, other studies are required to explain the effect of methotrexate on male and female reproductive systems and compare its effect with the use of natural substances that include *Boswellia carterii* and *Senegalia sengal* (gum Arabic).

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the figures in the manuscript are ours. Furthermore, figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No human studies are present in the manuscript
- The author(s) have signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' contribution statement

B.A.J was responsible of design, acquisition of data, organized research idea, and contributed to the paper writing. Statement A.H.A was responsible of design, conducted the revision and proofreading of the manuscript.

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علاج التهاب المفاصل الروماتويدي المستحث بواسطة نبات العلك المر والصمغ العربي في ذكور الفئران

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الملخص

التهاب المفاصل الروماتويدي (RA) هو مرض مناعي ذاتي يؤثر في الغالب على المفاصل الزليلية ويسبب التهابًا مستمرًا بالإضافة إلى تورم مؤلم يؤدي في النهاية إلى تقدم تدهور العظام والغضاريف مما يؤدي إلى تآكل العظام وتشوه المفاصل. هدفت الدراسة لمقارنه تاثير الصمغ العربي والعلك المرمع عقار الميثوتراكسيت لعلاج أعراض التهاب المفاصل المستحث في ذكور الجرذان بطريقة استخدام Complete Freund's adjuvant في مفصل الركبة اليسرى. تم أخذ مجموعة مكونة من 30 جرذ ابيض ذكر، ثم تم تقسيمها إلى ست مجموعات كل مجموعه بواقع 5 جرذان ذكور لكل مجموعة. وكانت المجموعة الأولى مجموعة السيطرة السالبة. (الجرذان غير المصابة بالتهاب المفاصل المستحث وبدون علاج) المجموعة الثانية هي مجموعة السيطرة الإيجابية (الجرذان المصابة بالتهاب المفاصل المستحث) المجموعة الثالثة (مجموعة التهاب المفاصل المستحث والمعالج بـ 250 ملغم / كغم من العلك المر) المجموعة الرابعة (مجموعة التهاب المفاصل المستحث وعولجت بجرعة 400 ملغم/كغم من الصمغ العربي) والمجموعة الخامسة (الجرذان المصابة بالتهاب المفاصل المستحث والمعالجة بالعلك المر والصمغ العربي) والمجموعة السادسة والأخيرة من التهابات المفاصل المستحث والمعالجة بـ 0.75 ملغم/كغم من عقار الميثوتركسيت. بعد ستة أسابيع من العلاج تم قتل حيوانات التجربة وأخذ أنسجة مفصل الركبة الأيمن والأيسر لدراسة التغيرات النسيجية في المفصل المصاب والمعالج .اوضحت نتائج الدراسة النسجية باستحداث التهاب المفاصل في الجرذان وجود التهاب في المفصل مع تورم واحتقان وتضخم في المفصل في مفاصل الحيوانات بينما اوضحت الدراسة ان الحيوانات المجرعة بالعلك المر والصمغ العربي ادى الى تقليل التغيرات الالتهابية في المفصل بالمقارنة مع عقار الميثوتراكسيت مما بين ان استعمال مواد طبيعية كانت ذات تأثيرات لتقليل اعراض التهاب المفاصل مع عدم وجود التأثيرات السلبية التي يحدثها عقار الميثوتراكسيت.

الكلمات المفتاحية: التهاب المفاصل، الصمغ العربي، مفصل الركبة، العلك المر، الميثوتراكسيت.