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ORIGINAL ARTICLE

Anti-inflammatory Effects of Hydro-Alcoholic Extracts of Mangrove (*Avicennia marina*) and vitamin C on Arthritic Rats.

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ABSTRACT
Rheumatoid arthritis is a common disease in today's society.mangrove (Avicennia marina) has long been used to reduce arthritic pain. Vitamin C as an antioxidant is effective in reducing symptoms of rheumatoid arthritis. This study was designed to investigate the effects of mangrove extract with vitamin C in a rat model of rheumatoid arthritis. To do this, 36 rats in 6 groups(healthy, arthritic, arthritic treated with 200mg/kg of the extracts, arthritic with 400 mg/kg, arthritic treated with Vitamin C+400mg / kg extracts and arthritis were treated with vitamin C) were distributed. Rheumatoid arthritis was induced by injection of complete Freund's adjuvant. **:**
The results showed that the group treated with high dose of vitamin C and high dose of extract have greatest impact on reducing inflammatory markers and improving joint lesions and the effect of high-dose groups treated with the extract plus vitamin C was not as well as previous groups.
Key words: *rheumatoid arthritis, complete Freund's adjuvant, mangrove plants, rat*

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease with symptoms of joint inflammation that affects approximately 1% of world population, it has been reported the incidence of the disease is three times more often in women than men [25]. Currently, there are two ways of medication therapy for this disease including nonsteroidal**,** anti-inflammatory drugs**,** and antirheumatic drugs [21]. Most medications used for treating RA, typically control pain and reduce inflammation or synovial joints and have a little effect on the processes of immune-inflammatory diseases. Therefore, these drugs cannot stop the progression of the disease and prevent the incidence of deformities in the joint cartilage and bone [5]. In addition, long-term side effects of taking these drugs, they are toxic and treatment costs with these drugs are also too high [9].Considering the disadvantages of many drugs used in recent years, the uses of herbs to treat patients with rheumatoid arthritis have focused by many research communities [16).

Avicennia or Mangrove plants grow in wet soils, tidal areas, wetlands and streams. This plant is growing in the Sea of Oman and the Persian Gulf. According to some old traditions, this plant has been used to treat various diseases for centuries. Varieties of mangroves due to compounds such as Steroids, Triterpenoids, Saponins, Flavonoids , Alcohols and Tannins have therapeutic properties for cancer tumors, bacterial infections, viral diseases specially hepatitis B, oral ulcers, gastric ulcers, as well as to deal with diseases of the cardiovascular and is used to delay blood coagulation [24, 15].

Many reports on how to improve or prevent joint destruction in rheumatoid arthritis patients are presented and oxidative damage has critical role in this regard [2, 17]. It is proposed that Oxidative damage by reactive oxygen species (ROS) leading cause of imbalance between the formation and inactivation of these compounds. Radicals of oxygen and their derivatives can be fatal to cells. There are several defense mechanisms to protect cells against the normal damage caused by the continuous production of active forms of oxygen [14]. Polyphenolic compounds are one of the ways to reduce the harmful effects of these substances. In addition, polyphenols are important materials in mangrove plants. Moreover, Flavonoids are the largest group of polyphenols. Flavonoids, based on their molecular structure**,** include various groups such as Anthocyanins. It is known that consumption of

rich foods in compounds of polyphenols could increase levels of antioxidants in the blood. In this regard, consumption of these compounds has beneficial effects against oxidative damage. In recent studies, therapeutic and preventive aspects of these compounds in various diseases such as rheumatoid arthritis have been confirmed [19]. Furthermore, many studies have reported that those who had the highest intakes of vitamin C and vitamin E supplementation showed an inverse relationship with Rheumatoid Arthritis [22]. In this study, the effect of hydro alcoholic extract of mangrove and vitamin C on reducing degradation of joint tissue in rheumatoid arthritis is investigated.

Studies on same chemical constituents of mangrove plant and other plants that have impact on inflammation showed effect of Flavonoids, alcohols, tannins and Terpenoids have been identified on this disease.

MATERIALS AND METHODS

Experimental animals

Wistar rats with an average weighing approximately 200 g and age of about five weeks were purchased from Isfahan University of Medical Sciences. The standard of care includes proper ventilation, a 12-hour period of darkness and light, temperature (21-22°C) and free access to food and water was provided in the burrows of animals.

Preparation of extract

The leaves of Avicennia marina were prepared from Assalouyeh region and detection of plant leaves was done by an expert biosystematics. For the extraction, some sections of this plant were powdered by crushing machine to increase the level of contact with the solvent. Then, 100 grams of Avicennia marina powder was weighed accurately with a digital scale and was poured into a one-liter Erlenmeyer flask. In the next stage, about 350 -400 ml of 96% ethanol was added so that it covered the total of powder.

Erlenmeyer flask was placed on a shaker for 24 h, and the solution was filtered.75% ethyl alcohol was added to the residue remaining and was placed on shaker again for 24 hours and then was filtered. The Filtrate from the first and second stages was poured and mixed into a container. Then the solution was poured into the balloon of distillation device in vacuum at 50 ° C and 70 rpm rotation speed until 1.3 volume of the initial solution was concentrated(Laborata 4000 Heidolph,Germany). The Solution which was concentrated in three stages (once with 100 ml and twice with 50 ml of chloroform) was discounted. Finally, Solution obtained from the last step was dried under sterile conditions at temperature of about 50 ° C [8].

Design of Experiments

In this experiment, 36 male rats were distributed randomly in six groups. These groups consisted of healthy, arthritic, arthritic treated with 200mg/kg of the extracts, arthritic with 400 mg/kg, arthritic treated with Vitamin C+400mg / kg extracts and arthritis were treated only with vitamin C. Healthy and arthritic control groups were treated only with saline solution and treated groups received the daily dose of extract by gavage for 30 days. Vitamin C in a dose of 50 mg per kg of body weight per day was placed in drinking water for the group.

Histopathological examination

Joint of the right foot of rat was isolated from the rest. The muscle tissue was removed and it fixed in formalin10%. After complete fixation, thin sections were prepared from tissues. Xylol and Hematoxylin – Eosin was used for clearing and coloring respectively. The slides were examined by microscope.

Review of Radiology

The right foot joint radiographic image of arthritic rats was taken (by Siemans-60MA, Germany) so that the extent of bone injuries could be examined.

Pathology Results

In slices prepared from normal group, synovium layer is white, free of granuloma inflammatory cells, without neutrophils, and lymphoplasma cells. The joint space of synovium is one layer and normal (Fig 1A).In tissue slices of patients, the synovium become inflamed, cells in synovial increase (synovium hyperplasia), joint space narrowing, inflammatory cells, mononuclear (macrophages, plasma cells and lymphocytes) and granuloma Panus is clear (Fig1. B, C).In the group that treated with 200 mg of extract not visible significant improvement compared to patients.In histological slices of this group, extensive reactions granulomatosis with acute and chronic inflammatory cells, synovial

hyperplasia and joint space narrowing is seen (Fig1. D, E).In rats treated with 400 mg of extract**,** significant difference was observed in patients. Also, Joint tissue is without inflammatory cells, Granulomatosis reactions, synovial hyperplasia and panus were not found. Joint space is normal. Only a few inflammatory cells were seen in the soft tissue (Fig1. F). Groups treated with doses of 400 extracts with vitamin C shows granulomatosis reactions similar to patients' groups. Moreover, the presence of inflammatory cells, mononuclear and lymphoplasma cells, the panus and narrowing of the joint space in tissue sections is clear (Fig1. G, H). In the group treated with vitamin C**,** inflammation and granulomatosis reaction were seen less than those in patient groups .There is not Panus, joint space is normal, but synovial hyperplasia with lymphoplasma cells were seen (Fig1.I).

RESULT

X-rays were taken of joints and paws rats to examine the extent of damage and change in bones. In arthritic group**,** deformed bones of paws, muscle inflammation with arthritis of the fingers when compared to the controls were observed (Fig. 2B). Also, we didn't observe any significant difference in the treatment of 200 mg/kg of extracts with patients (Fig. 2C). In the group treated, it was observed 400 reduced inflammation of muscles and joints of the toes (Fig. 2D). There was not a visible difference in toe arthritis and inflammation of muscle tissue in treated group with 400+Vitc than those in the group of patients (Fig. 2E). Moreover, you can see reduction in damage of bones and deformation of bones in treated group of vitamin C (Figure.2F).

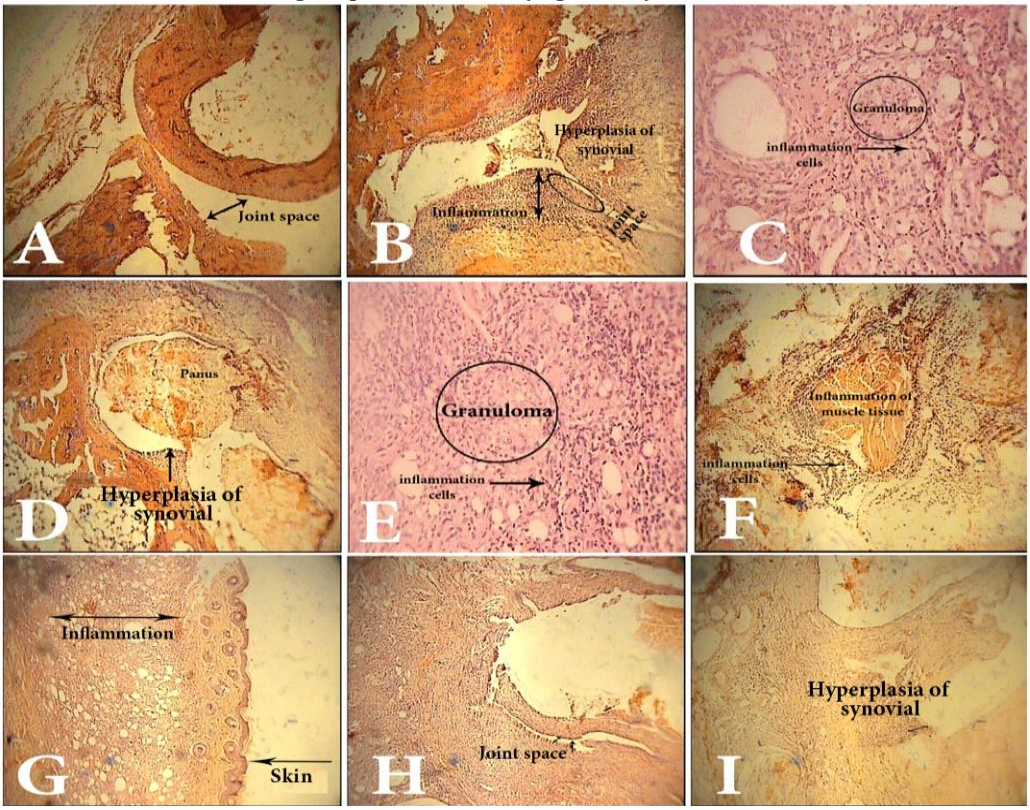

Figure 1: Pathological Pictures of the different groups. A: Joints cross section of controls with magnification 10x. B: Joints cross section of patients with magnification 10x. Hyperplasia of synovium and joint space narrowing was observed. C: Joints cross section of patients with magnification 40x. Granulomas and mononuclear inflammatory cells (macrophages, lymphocytes, and plasma cells) were visible. D: Joints cross section of the group treated with 200mg/kg of the extracts (magnifications 10x). Hyperplasia of synovium and Panus were observed. E: Joints cross section of the group treated with 200mg/kg (magnification 40x). Granulomas and mononuclear inflammatory cells (macrophages, lymphocytes, and plasma cells) were detected. F: Joints cross section of the group treated with 400mg/kg(10x). Inflammation was seen only in soft tissue. G,H: Joints cross section of the treated group by vitC plus 400mg/kg(10x). Reactions granulomatosis and joint space narrowing was observed. I: Joints cross section of group treated with vitC (10x). Inflammatory cells (lymphocytes) and synovial hyperplasia was observed.

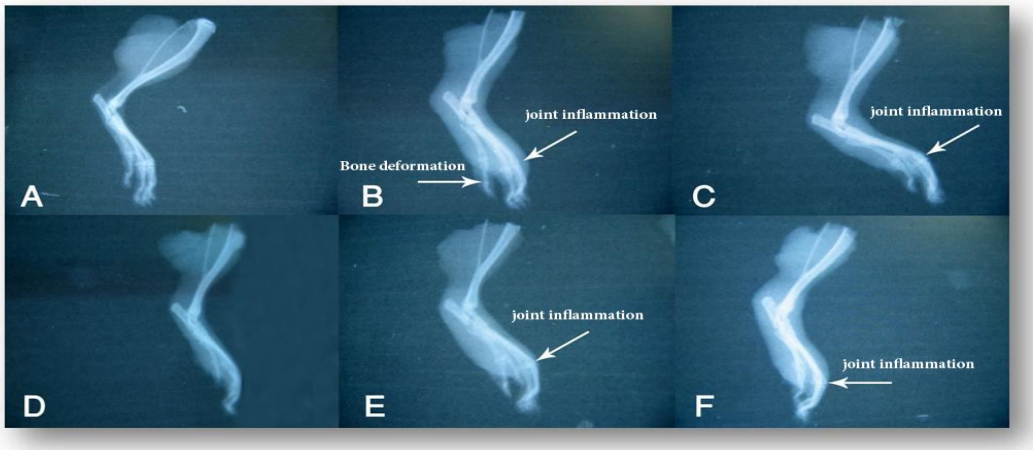

Figure 2: Photographs of the different groups examined radiologically. A: healthy group. B: patient group, arthritis and bone deformation was observed. C: Group treated by 200 mg/kg of the extracts, joint inflammation was seen such as patient group. D: The group that treated by400 mg/kg of the extracts. E: Treatment vitC + 400 mg/kg, joint inflammation was observed .I; Treatment vitC, Joint inflammation was seen less than patient.

DISCUSSION

In various studies, different animals such as rabbit [3], horse [23] and rat [10] has been used to create models of rheumatoid arthritis. The most Similar to the model of human chronic inflammatory regarding Histopathology is arthritis induced in rats.Arthritis like human RA can occur with stimulation of the immune system by the injection of complete Freund's adjuvant in rats [7, 6] which were used in this study.

In the present study, a model of RA was created by injecting 0/05% ml complete Freund's adjuvant (CFA) with a concentration 5mg/ml in the right hind paw of the rats. After the injection, the signs of inflammation were seen such as: redness, swelling and hardening joints. Nearly, diameter of joint swelling peaked in between 13th and 16th days and this swelling remained up to 30 days [11, 4]. This model Prepare the best time for doing experiment periods, chronic inflammation and also observing rate of recovery by medicines. It consists of two phases that the initial injuries in the acute phase usually occur in the seventh day after adjuvant injection. Histamine and serotonin in the initial phase and prostaglandin, protease and Lysosome in the second phase usually released. In the second phase, secondary damages visible and distinguishable after 21 days of adjuvant injection. Usually, the second phase more sensitive to anti-inflammatory drugs [20, 18]. As stated earlier, the genus Avicennia has chemical compounds such as Flavonoids, Alcohols etc. that these compounds have anti-inflammatory effects and reduce the destruction and injury of rheumatoid arthritis [24]. Furthermore, Specific studies on Flavonoid obtaining from various plants identify that Flavonoids reduce levels of inflammatory markers, protect articular cartilage and they also effective in improving destruction rheumatoid arthritis in cartilage. It also stated that Flavonoids have potential role in clearing free radicals and they effectively reduce the production of NO (nitric oxide) , TNF-α and apoptosis and all these results verify the effects of reduction of degradation and increasing reconstruction of mangrove plant extracts.

There is no significant difference in the group treated by 200 mg/kg of the extract in comparing with those in control group. Perhaps, the main reason is low dose of extract. The rats which received only vitamin C, inflammatory factors significantly decreased when compared with those in control patients. According to studies on the anti-inflammatory properties of vitamin C, it can be said that Vitamin C in the first stage increases the level and retrieval of antioxidants such as vitamin E. In the next stage, antioxidants such as vitamin C can reduce the level of ROS (Reactive oxygen species). Regarding the mechanism of action of vitamin C studies have shown that ROS causes degradation of cartilage by either inhibition of matrix production, cell migration, activity of growth factors or directly decomposition of component matrix. Vitamin C as an antioxidant and as a reduction of lipid peroxidation can decrease the level of it, and it maintains the development and production of natural bones and cartilages [13, 1] .In addition, vitamin C probably decreases the destruction of bone and

cartilage through inhibition of cyclooxygenase , inhibition of production of PGE 2**,** and the reduction level of NO [12].

In the group treated by 400mg/kg extracts together vitamin C, we could see a significant reduction in inflammatory markers compared with control arthritic. On the contrary, a lower level of inflammatory factors was observed in compare to the groups received doses of 400 extracts and vitamin C. Maybe the reason is the synergistic effect of mangrove extract and vitamin C.

CONCLUSION

In this study**,** it was shown the active compounds in extracts of mangrove in high doses have a positive effect on reducing inflammation caused by rheumatoid arthritis. In addition, this reduction is more than chemical antioxidants such as vitamin C. However, the same dose of active substances with vitamin C is not a desirable outcome.

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