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Anti-Arthritic Effects of Moringa Oleifera and Vitamin D on Articular Cartilage; A Comparative Study

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ABSTRACT

Objective: To compare the anti-arthritic effects of Moringa Oleifera and Vitamin D on articular cartilage of formalin induced arthritic rat model.

Methodology: This experimental, comparative using an animal model study was conducted from September 2020 to September 2021 in the department of Anatomy at Islamic International Medical College Rawalpindi in collaboration with the National Institute of Health, Islamabad. Forty adult male Sprague Dawley rats were divided into four groups each containing 10 rats. All animals except group A received sub planter injection of formaldehyde on right paw on day 1 and day 3 of experiment. Group A, control and group B, negative control group were given standard rat diet. Male rats of treatment group C were given 4000IU/kg body weight of vitamin D and those of group D were given 500 mg/kg body weight of Moringa aqueous extract throughout the experiment via oral route. At the end of experiment, the right hind limb was removed, processed, and stained by H&E and toluidine blue. Results were analyzed using SPSS version 21.

Results: The histomorphological changes were evaluated according to OARSI (Osteoarthritis research society international) grading of osteoarthritis (OA). Moringa treated group exhibited grade I&II and while Grades II, III & IV were revealed in vitamin D treated group.

Conclusion: Moringa oleifera was found to be an effective antiarthritic agent compared to vitamin D on histomorphological changes of articular cartilage in formalin induced arthritic rat model.

Keywords: Moringa, Vitamin D, Articular cartilage, Osteoarthritis, Formaldehyde induced arthritis, Flavonoids.

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Introduction

The most prevalent rheumatic illness, osteoarthritis (OA), usually manifests in old age. In women, the prevalence is 13%, whereas in men over 60, it is 10%. Although it usually worsens with age, it can also affect younger people.[1] Many times, osteoarthritis is described as a wear-and-tear disease. The articular cartilage is present on the joint surface and is divisible into four zones. The superficial zone which is composed of 10% to 20% of total cartilage, then there is an intermediate zone which is present deep to the superficial zone. It is the thickest zone forming about 40% to 60% of the total cartilage volume.

The deep (radial) zone lies below the transitional zone and represents approximately 30% of articular cartilage volume. The calcified zone contains spherical chondrocytes embedded in a calcified matrix. The calcified zone is separated from the deep (radial) zone by calcified line called the tidemark.[2] The OA leads to degradation of cartilage and bone remodeling due to active response of inflammatory cells and chondrocytes. The cartilage damage due to release of enzymes from these

cells cause breakdown of collagen and proteoglycans causing deterioration and thinning of articular cartilage.[3] There are four stages of osteoarthritis (OA), with stage zero being a normal, healthy joint and stage four indicating an advanced, severe case of the disease.[4]

Vitamin D deficiency significantly correlates with OA incidence and progression, suggesting that supplementation can prevent the development and progression of OA by enhancing bone and cartilage biological functions.[5] Research shows that vitamin D supplements improve arthritic rats' articular cartilage thickness, extracellular matrix fiber deposition, and joint lubrication. Vitamin D3 is prepared in the skin from 7-dehydrocholesterol, which undergoes two hydroxylation processes in the liver and kidney to become active. The pathophysiological role of vitamin D metabolites in osteoarthritis (OA) is reflected by the presence of vitamin D receptors (VDR's) in bone, cartilage, and muscle. Vitamin D's binding to VDRs inhibits the Wnt/β catenin signaling pathway.[6] This reduces bone turnover and cartilage degeneration, hence reducing the advancement of OA. Inadequate serum vitamin D levels have a negative impact on calcium metabolism and articular cartilage turnover.[7]

Moringa oleifera (MO), often known as the drumstick tree or horseradish tree, originated in Northern India and Africa and has been studied for its potential health benefits.[8] Moringa leaves, a vital plant source of nutrition, are rich in calcium, vitamins, carotenoids, protein, iron, potassium, polyphenols, flavonoids, and antioxidants, providing significant osteoprotective benefits. MO leaf extracts, particularly its bioflavonoid Kaempferol, showed in vitro free radical scavenging, anti-inflammatory, and anti-arthritic properties. [9]

In an arthritic rat model, Moringa's aqueous extract restored cartilage damage. Based on these facts, this study was designed to compare the effects of Moringa oleifera and vitamin D on an arthritic rat model.

Methodology

The experiment was conducted in a laboratory, and the sample was drawn using a non-probability consecutive sampling procedure. This study was conducted from September 2020 to September 2021 in the department of Anatomy at Islamic International Medical College Rawalpindi in collaboration with the National Institute of Health (NIH), Islamabad following approval from the Ethics Review Committee [Riphah/IRC/20/243].

The study used 30 Albino Sprague Dawley adult male rats as a mammalian model. The study included two-month-old adult male rats weighing 300gm, while rats weighing less than 300gm and female rats were omitted.[10] The animal care and handling was carried out in accordance with the rules established by the Ethical Review Committee of Islamic International Medical College, Rawalpindi.

The MO leaves were thoroughly cleaned with tap water to remove all contaminants and debris. The washed leaves were dried in the shade and then finely ground in a grinder and sieved. In our laboratory, 100gm of dry leaf powder was mixed with 1000ml of boiling water for five minutes to make the aqueous extract.[12] The mixture was filtered through a sterile filter paper and placed in a sterile tube. Each set of studies used a newly produced aqueous extract containing 100 mg of MO/ml. It was then stored at 4°C for six days. MO leaves were purchased from local herbal store. Vitamin D injections were acquired from a local pharmaceutical company.

Rats were maintained in cages under the supervision of the National Institute of Health's Animal House in Chak Shehzad Islamabad. Forty-five rats weighing roughly 300gm were housed under standard temperature at 22 ± 0.5˚C in an air-conditioned room. They were then placed into clean stainless-steel cages under a 12-hour light and dark cycle with 50% humidity.[11] They were fed and watered ad libitum for seven days to acclimatize. Each group consisted of ten male rats.[13]

Rats in Control Group A were kept on a standard diet orally throughout the experiment for 28 days. Rats in Negative Control Group B were also kept on a standard diet orally throughout the experiment for 28 days. In Treatment Group C, the rats were given 4000 IU/kg of vitamin D orally by adding cholecalciferol (Vitamin D3) injection to their standard diet for 28 days. Rats in Treatment Group D received an aqueous extract of Moringa oleifera (MO) leaves at a dosage of 500 mg/kg orally mixed in their diet for 28 days

Arthritis was produced one hour after subplantar administration of 0.1 ml formaldehyde (2% v/v) into the left hind paw of all the animals on days 1 and 3 of experiment. After 24 hours of the last medication, the rats were anaesthetized using chloroform-soaked cotton balls until they lost consciousness (euthanasia). Afterwards the right hind leg was removed using a bone cutter just proximal to the ankle joint. Then it was cleaned with saline and preserved in 10% formalin. Formic acid was used to

decalcify the bone over a 10-day period. The ankle joints after decalcification were separated into equal parts, embedded in paraffin wax, serially sectioned at 200mm intervals, and microtome-cut into slices for histopathological examination. The slide were stained with hematoxylin and eosin (H&E) and toluidine blue (T-blue).[12] The histological alterations in articular cartilage were graded using the Osteoarthritis Research Society International (OARSI) ratings. The OARSI grading (0-5) is based on joint surface changes, both horizontally and vertically. (Table I) The data was entered and evaluated using SPSS version 21, and the findings were given as mean ± SD. The mean thickness was compared between the treatment, negative control, and control groups using one-way ANOVA. The post hoc Tukey's test was used to compare the groups. A p-value of <0.05 was considered significant.

Table I: OARSI (Osteoarthritis research society international) grading[14]

Grade	Features	Zone involved
0	Cartilage morphology intact	No zone involved
1	Surface intact with superficial fibrillations, hypertrophy of chondrocytes and cell apoptosis (empty lacunae) Minimal cartilage degradation (damage) 5 -10%	Surface zone
2	Deep fibrillations in superficial zone with chondrocyte hypertrophy and clustering. Mild cartilage degradation 11-25%	Surface zone extending to upper part of midzone
3	Vertical fissures extending to midzone and upper part of deep zone and pannus formation Moderate cartilage damage 26-50%.	Mid zone
4	Cartilage erosion with matrix loss extending to deep zone and calcified cartilage. Marked cartilage damage 51-75%	Deep zone and calcified cartilage
5	Denudation having complete loss of cartilage with bone sclerosis and micro fractures with foci for fibrocartilage repair Severe damage > 75%	Calcified cartilage and subchondral zone

Results

The control group had an undamaged surface with minimal cartilage injury (OARSI grade I). The negative control group B demonstrated almost complete loss (erosion) of uncalcified cartilage, with damage extending to calcified cartilage, resulting in bone with micro fracture of the subchondral bone plate in approximately 60.0% of rats (OARSI grade = 5). The remaining 40.0% of rats in group A showed cartilage deterioration with profound cracks that extended into the deep zone. Chondrocyte apoptosis (empty lacunae) and damaged cartilage remains were observed in the joint space (OARSI grade = 4). P-value < 0.01. (Figure 1)

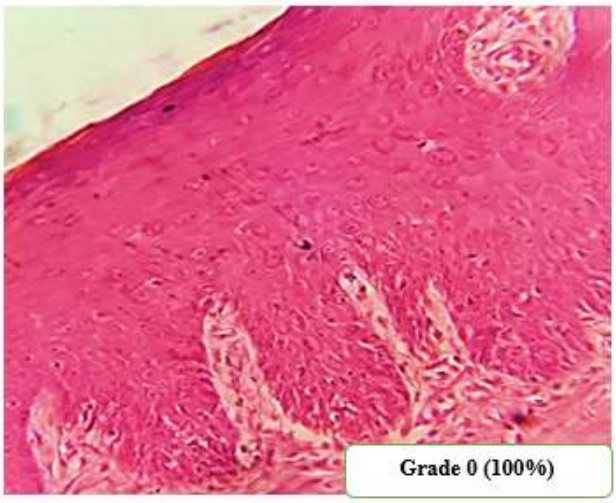

Figure 1:Control group A having intact cartilage with no damage (OARSI grade=0)

Surface discontinuity with fibrillation extending across the superficial zone was observed in 50% of rats from group B. (Figure 2)

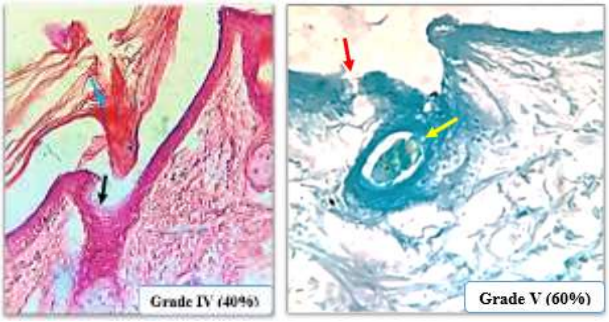

Figure 2. Photomicrograph of coronal section of ankle joint of group B showing vertical fissures extending in deep zone and calcified cartilage, OARSI grade=4 in 40% rats. (40x10x H&E). Cartilage erosion with bone denudation, micro fracture, and fibrocartilage repair. OARSI grade=5 in 60% of rats. (Toluidine blue stains at 40x10x). Red arrows show bone denudation and micro fractures in toluidine blue. Black arrows showed deep vertical fissure. Blue arrows showed damaged cartilage remnant. Yellow arrows showed fibrocartilage repair.

Some fibrillations were noticed extending into the midzone, but no damage was seen there. Hypertrophied chondrocyte clusters were observed in the superficial and middle zones (OARSI grade = 2). In this group, 30% of the rats had an intact articular cartilage surface, with few empty lacunae and hypertrophied chondrocytes in the

surface zone. Surface fibrillations were also observed in the superficial zone (OARSI grade = 1). The remaining 20% of rats in group B showed deep vertical fissures and fibrillations extending to transitional zone (mid zone) some fibrillation in deeper part of mid zone. Cellular death and apoptosis were observed near cracks, as well as cartilage matrix degradation (OARSI grade=3) P-value < 0.01. (Figure 2)

In group C, 70% of the rats showed only surface fibrillations and abrasions with hypertrophied chondrocytes in superficial zone (OARSI grade I). Remaining 30% of rats showed deep fibrillations involving superficial zone with hypertrophied chondrocyte clusters near fibrillations (OARSI grade II) P-value < 0.01. (Figure 3)

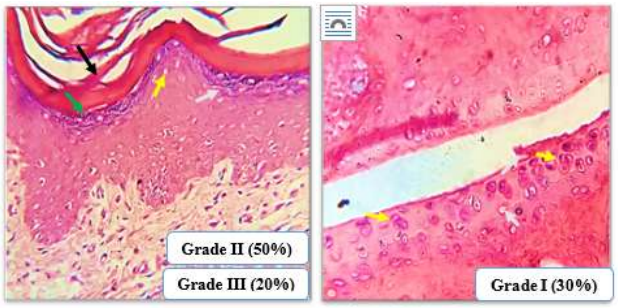

Figure 3. Photomicrograph of coronal section of ankle joint of group C showing deep fibrillations (fissures) extending in midzone in 20% (OARSI grade=III), surface fibrillations in 30% (OARSI grade=I) and chondrocytes hypertrophy and clusters in superficial and middle layers in 50% (OARSI grade=II). (H&E stains at 40×,10x). Yellow arrows show chondrocyte clusters. Green arrows showed deep fibrillations. Black arrows show pannus. White arrows showed empty lacunae.

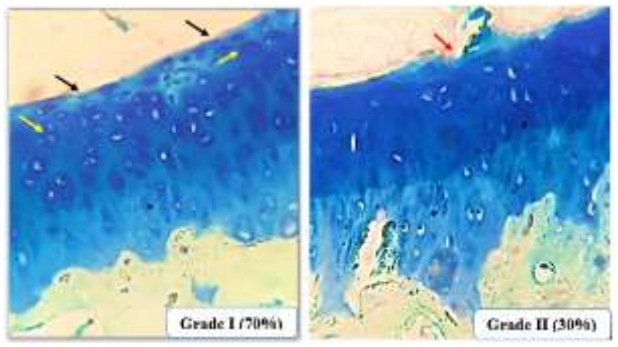

Figure 4. Photomicrograph of coronal section of ankle joint of group D showing superficial fibrillations, chondrocytes hypertrophy and clusters in superficial layers (I & II). OARSI grade=1 in 70% rats. 30% shows deep fibrillations (III) OARSI grade=2. (Toluidine blue stains at 40x). Yellow arrows show chondrocyte clusters. Black arrows show surface fibrillations (I, II). Red arrow shows deep fibrillations.

Discussion

Osteoarthritis is a form of joint degeneration that usually impacts the lower limb. It affects joint flexibility, making movements uncomfortable.[15] Depending on the severity of the ailment, effective osteoarthritis care may consist of a combination of lifestyle modifications, drugs, and other therapies. Vitamin D supplementation is often suggested for OA due to its advantageous impact restoring cartilage damage.[16] Because of the hazardous side effects and high cost of pharmaceutical medications, herbal drugs are gaining popularity as a safe and efficient arthritis treatment. Moringa extract leaves are used for alleviating inflammation as well as pain caused by several degenerative illnesses.

There is limited research available on the influence of Moringa leaf extract and vitamin D on histomorphological changes caused by osteoarthritis, especially qualitative evaluations of articular cartilage thickness. So this study planned to investigate the antiarthritic properties of Moringa oleifera and vitamin D in an arthritic rat model. The dose of Moringa extract and vitamin D used throughout this trial was 500mg/kg and 4000IU/kg, respectively.[12]

This study has investigated the changes of articular cartilage in response to sub plantar administration of formaldehyde. OARSI grading system for cartilage histopathology was used to measure the articular cartilage degeneration in the three experimental groups.[17] In negative control group 60% of rats have deep fissures with cartilage erosion and bone denudation. Micro fractures of bone were seen in areas of cartilage erosion (OARSI grade=5). 33 40% of rats in this group showed cartilage erosion extending to calcified cartilage (OARSI grade=4). This is in accordance with the study done by Ikufumi Takahashi in 2017 and Iffah Nadhira in 2018.[18,19] In vitamin D treated group 20% rats revealed vertical fissures extending to midzone and pannus formation (OARSI grade=3). 50% rats revealed deep fibrillations in superficial zone with hypertrophied chondrocyte clusters. Empty lacunae were present in superficial and transitional zone (OARSI grade=2).101 30% rats showed surface fibrillations with intact cartilage surface (OARSI grade=1). Cecilia Pascual-Garrido in 2016 reported that vitamin D deficiency caused adverse changes in hyaline cartilage of normal healthy rats.[20] Lower serum levels of

vitamin D was associated with increased chondrocyte clustering and cartilage matrix loss in arthritic knee joint.[21]

Group treated with Moringa extract showed superficial fibrillations and few hypertrophied chondrocytes with intact cartilage surface in 70% rats (OARSI grade=1). Remaining 30% revealed deep fissures in superficial zone with hypertrophied chondrocyte clusters (OARSI grade=2). This is in agreement with an international research work done by Ammara Saleema in 2019 that reported significant improvement in inflammation and erosion of cartilage in the arthritic rat model treated with 600mg/kg aqueous extract of Moringa.[22] Presence of phenolic compounds in MO was responsible for its anti-inflammatory properties was revealed in the review articles by Mariateresa Maldini in 2014 and Thulani Tshabalala in 2019.[23] A study done in 2018 revealed that Moringa leaves possessed high concentrations of quercetin and kaempferol that prevented nuclear factor kappa B (NFkB) dependent inflammation in mice.[24] No evidence is available regarding effect of Moringa extract on OARSI histological grading in arthritic rat model. The findings support the use of this plant extract as a medication to treat the pain and inflammation associated with OA.

However, this experimental study has few limitations. Surgically-Induced OA Models of knee joint are excellent for short term studies but it is costly and needs more skill and equipment. The study did not determine the changes at the molecular level. Further research could explore the effect of MO and vitamin D on histopathological changes in synovial membrane and bone.

Conclusion

In conclusion, Moringa oleifera was demonstrated to be a more effective antiarthritic agent than vitamin D in reducing the histomorphological alterations of articular cartilage in an arthritic rat model. This study also investigated the therapeutic and nutritional attributes of Moringa Oleifera leaves in an arthritic rat model.

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