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Original Article

Anti-Inflammatory Effect Of Moringa Oleifera And Vitamin D On Articular Cartilage Degradation In Arthritic Rat Model: A Comparative Study

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ABSTRACT

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

Study Design: Laboratory based experimental study Its lab based experimental study in which the effects of two drugs are compared there is no term comparative

Place and Duration of Study: Conducted in collaboration with the National Institute of Health (NIH) and the Department of Anatomy at Islamic International Medical College from September 2020 to September 2021.

Methodology: as heading patients and method cannot be used here so written methodology Forty mature male Sprague Dawley rats were put into four groups of ten each. Group A, control and group B, negative control group (the untreated arthritic rat 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. On the first and third days of experiment, all animals received subcutaneous injections of formaldehyde into their right paws except the control group A. The rat's body weight was measured and recorded on day 1, 14 and 28. After the 28-day trial period, the right hind limb was removed, processed and stained with H&E for microscopic examination.

Results: The Moringa aqueous extract administration significantly reverted the articular cartilage damage when compared with vitamin D (p-value <0.001 on intergroup comparison) in formaldehyde induced arthritic rat model. Changed it in this document accordingly results explained statistically

Conclusion: Moringa Oleifera, a plant known for its anti-inflammatory properties, was found to be more effective than vitamin D in repairing articular cartilage in an arthritic rat model. Word count reduced

Keywords: Cartilage, formaldehyde-induced arthritis, flavonoids, Moringa, osteoarthritis, vitamin D.

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INTRODUCTION

The most common form of joint degeneration is osteoarthritis (OA), which causes tenderness and permanent damage to the joints. The molecular processes of osteoarthritis are unknown.1 OA is an intricate medical condition that affects the entire joint, involving an array of tissues as it progresses. It corresponds with the risk of limited mobility (defined as needing help walking or climbing stairs) for people with damaged knees that are larger than that caused by any other illness in adults aged 65.[2]

Osteoarthritis, which was formerly assumed to be a degenerative 'wear and tear' joint ailment affecting cartilage and underlying bone, is now classified as an inflammatory disorder. Vitamin D insufficiency is quite common in osteoarthritis patients, and it is linked to increased joint pain and functional impairment.[3] Vitamin D reduces bone turnover and cartilage degeneration, which may postpone the onset and progression of OA.[4] Natural foods have minimal amounts of vitamin D, whereas several are fortified. Supplements are the most effective means to obtain it; they come in two forms: vitamin D2 and vitamin D3, both of which are naturally occurring generated by the sun's ultraviolet-B (UVB) rays, thus the name "sunshine vitamin." Many research investigations have discovered that vitamin D receptors (VDR) exist in most of the body's tissues and cells, including chondrocytes. However, the severity of OA directly affects the function of vitamin D receptors (VDR) in various kinds of organs. VDR modulate vitamin D concentrations in chondrocytes, resulting in an even oxidative environment.[5]

Moringa Oleifera (MO) is a tree with rapid growth and resistance to drought native to the Indian subcontinent that is extensively utilized in South and Southeast Asia. Almost every part of the tree is eaten

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or utilized to make traditional herbal medicines.[6] This is especially true for the leaves, which are commonly consumed in parts of India and Africa. MO offers a steady stream of potentially beneficial compounds. It is composed of various distinct chemicals. These include glucosidases, polyphenols (phenolic acids and flavonoids), tannins, vitamins, saponins and minerals. Flavonoids have significant antioxidant properties and transfer electrons or hydrogen atoms via metal chelation. MO methanolic and ethanolic leaf extract includes anti-inflammatory effects, targeting superoxide and peroxyl radicals, consequently it is advised for use in8. degenerative inflammatory illnesses.[7]

In an arthritic rat model, Moringa aqueous extract reversed cartilage deterioration.[8]. Based on these findings, the current study sought to investigate the effects of Moringa Oleifera and vitamin D on an arthritic rat model.

METHODOLOGY

It was a laboratory-based experimental comparative study Again lab based study as already explained conducted in collaboration with the National Institute of Health (NIH) and the Department of Anatomy at Islamic International Medical College after approval from the Ethics Review Committee, from September 2020 to September 2021 (Riphah/IIMC/IRC/24/1031) Irc no added. A convenient random selection was performed by randomly placing rats in different cages. The sample size was determined by the resource equation method24. Reference added for sample size as in random sampling no other methodology involved. The mammalian model employed in the study was 40 adult male Albino Sprague Dawley rats.

Inclusion criteria: The study comprised two-month-old adult male rats weighing 300gm.

Exclusion criteria: Rats weighing below 300 grams and female rats were excluded.

Moringa leaves were thoroughly cleansed with tap water to eliminate dirt and pollutants, followed by drying in the shade and powdered in grinder and filtered. The plant aqueous extract was prepared by mixing 100gm of dry leaf powder with 1000ml of water that had been boiled in laboratory for five minutes. The resulting mixture was filtered using sterile filter paper and placed into a sterile tube8. Each set of experiments used a newly produced aqueous extract with 100 mg of MO/ml. MO leaves were sourced from a nearby herbal store. Vitamin D injections were obtained from a local pharmaceutical company.

The National Institute of Health's Animal House in Chak Shehzad Islamabad kept rats in cages under their supervision. In an air-conditioned room'40 rats weighing approximately 300gm were kept at the standard temperature of 22± 0.5C. They were then placed in spotless steel cages with a 12-hour light/dark cycle and 50% humidity. They were fed and hydrated liberally for seven days to acclimatize.[9] Each group contained 10 male rats. Control group A, Negative control group B and two treatment groups C&D for 28 days, Group A (control) and Group B (negative control) consumed a regular meal orally. For 28 days, rats in group C were provided with vitamin D 4000IU/kg orally by adding cholecalciferol (Vitamin D3) into their regular meal Rats in group D were given aqueous extract of MO leaves 500mg/kg orally mixed in diet for 28 days. On day 1 and 3 of the experiment, arthritis was induced by sub plantar injection of 0.1ml of formaldehyde (2% v/v) into all of the animals except control group A. The negative control group B is untreated arthritic rat group given standard diet like control group A. This was done one hour after the vehicle/drug had been administered orally. The rats were exterminated 24 hours after receiving the prior dose by putting them to sleep with chloroform-soaked cotton balls [10].

The animals' body weights were recorded with a digital balance on the first day after induction, the 14th day, and the end of the 28th day. The right hind limb was then severed using a bone cutter around the ankle joint. Then is cleaned with saline and kept in 10% formalin. Over 10 days, the bone was decalcified using formic acid. Following decalcification, the ankle joints were separated into equal parts, submerged in paraffin wax, serially sectioned at 200mm intervals, and microtome-sliced for histological examination. The slides were stained with hematoxylin and eosin (H&E). To assess cartilage damage depth, the articular surface of the talus was separated into three equal zones. Zone 1 was on the outer edge adjacent to the capsule, followed by zone 2 and zone 3 were on inner side (figure 1). A line was drawn parallel to the cartilage surface, and depth was measured in three zones, separated by four parallel lines drawn perpendicular to the cartilage. Cartilage depth was measured from the midpoint of each zone by drawing a line from surface to cartilage depth [11]. The depth in

each zone was computed using image J software, and the average depth of cartilage damage was determined.[12]

The data was entered and evaluated using SPSS (Statistical Package for the Social Sciences) version 21. Results are reported as Mean±Sd. A one-way ANOVA was performed to compare the mean thicknesses of the treatment, negative control, and control groups. The post-hoc Tukey's test was used for comparing the groups. *p*-values below 0.05 were regarded as significant.

RESULTS

A measurement of weight by digital balance revealed that administration of Moringa and vitamin D cause statically significant weight gain in group C and D when compared with negative control group B (*p*-value <0.01 on intergroup comparison). On day 28 the mean body weight was found to be 246.3±0.6gm, 161±0.6gm, 278.1±0.6gm and 302.7±0.6gm respectively. When the mean values of group C and D were compared weight gain is significant in group D as compared to group C. While the comparison of group B with group C and D were also found to be statistically significant indicating decrease in body weight in group B (*p*-value <0.01). No statistically significant difference was found after comparing group A with group C and D (table 1).

An examination of hematoxylin/eosin-stained ankle joint sections by microscopy revealed that administration of Moringa aqueous extract and vitamin D significantly reserved the cartilage damage by decreasing the cartilage depth in both treatment groups C&D (*p*-value <0.01 on intergroup comparison). The mean cartilage depth was found to be 0.00um, 47.5±0.8um, 26.9±0.6um, and 5.49±0.4um in control A, negative control B, and treatment groups C and D, respectively. On inter group comparison, when the mean values of group C and D are compared the group D showed significant decrease in cartilage depth as compared to group C (*p*-value <0.01). There was significant increase in mean values of cartilage depth in negative control group B when compared with control group A and treatment group C&D (*p*-value <0.01). Moringa oleifera and vitamin D restored the cartilage damage with Moringa being more effective than vitamin D (table 2, figure 1).

DISCUSSION

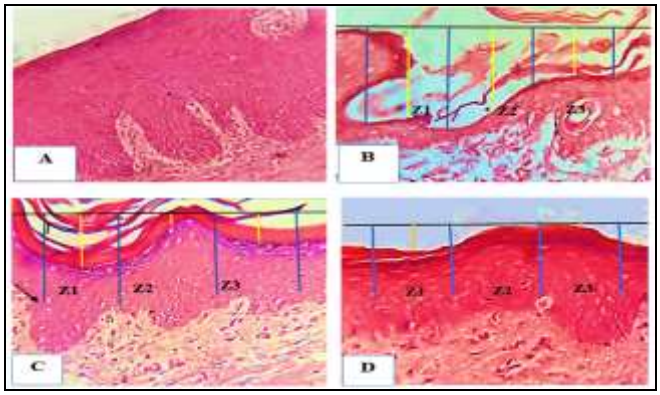
Osteoarthritis is a degenerative disease that causes loss of articular cartilage. It is the most common type of arthritis, causing functional and structural damage to the synovial joint. The disease's major course is cartilage weakening and loss. Vitamin D has a crucial role in maintaining calcium hemostasis and is thus needed for healthy bones and teeth5'13. Its anti-inflammatory properties aid in preventing joint degeneration in inflammatory joint diseases like OA. Because of harmful side effects along with the expansive cost of pharmaceutical treatments, herbal remedies are gaining popularity as safe and effective arthritis treatment. Multiple investigations have found

Table-I: Mean Measurement Of Body Weight±SD Gm Among The Control (A), Negative Control (B) Verses Treatment Groups (C&D) At Day 1, Day14 And Day 28

Parameter	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	Group D Mean±SD	*p*- value	
Day 1 (Body weight in gm)	246.3±0.62	246.4±0.62	246.3±0.62	246.2±0.62	*p*-value <0.01	
Day 14 (Body weight in gm)	246.3±0.62	179.8±0.58	274.1±0.68	297.1±0.72		
Day 28 (Body weight in gm)	246.3±0.62	161±0.52	278.1±0.71	302.7±0.75		
Intergroup comparison	A versus B *p*-value <0.01	A versus C *p*-value <0.05	A versus D *p*-value <0.09	B versus C *p*-value<0.01	B versus D *p*-value <0.01	C versus D *p*-value<0.01

Table-II; Mean Measurement of Cartilage Damage Depth±SD um Among the Control (A), Negative Control (B) verses Treatment groups (C&D).

Parameter	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	Group D Mean±SD	*p*- value	
Cartilage damage Depth±SD um	0.00	47.51±0.78	26.9±0.62	5.49±0.54	*p*-value <0.01	
Intergroup comparasion	A versus B *p*-value <0.01	A versus C *p*-value <0.01	A versus D *p*-value <0.09	B versus C *p*-value<0.01	B versus D *p*-value <0.01	C versus D *p*-value<0.01



Figure; Photomicrograph of Coronal Section of Ankle Joint Showing Cartilage Damage (depth) Indicated by yellow lines, in Negative Control b and Treatment group c & d. Group a Shows Intact Surface with no Damage. Zones are z1 (zone 1), z2 (zone 2) and z3 (zone 3) From lateral to medial

that Moringa Oleifera leaves have anti-inflammatory qualities[14].

Few studies are available revealing the effects of Moringa leaf extract and vitamin D on histomorphological changes of articular cartilage caused by osteoarthritis8. So, the purpose of this study was to inquire at the antiarthritic activities of Moringa Oleifera and vitamin D in arthritic rat model. The doses of Moringa extract and vitamin D administered during study were 500mg/kg and 4000IU/kg, respectively.

This investigation showed that the body weight of animals in negative control group declined significantly but reverted in vitamin D and Moringa treated group as shown in figure 1. The mean difference between the control group and Moringa treated group was minor but was significant when compared with vitamin D treated group as shown in figure 1. Aremu A carried out a study in 201815and discovered that the arthritic rat model lost weight after eight day due to the appetite loss caused by inflammation, pain and stress. This weight loss was ameliorated by a single intra-articular dose of vitamin D analogue calcipotriol, but in current investigation, oral treatment induced weight gain in the vitamin D treated group C[16]. This is most likely owing to its anti-inflammatory characteristics, which were revealed in numerous research projects as done by Garfinkel RJ in 2017 and Meireles in 202016'[17]. This study found a significant difference (p-value <0.01) between the vitamin D and Moringa treated groups on the 28th day, indicating that Moringa raised weight more effectively than vitamin D.

In arthritic rat model, oral consumption of Moringa improved nutrition absorption via the intestines, leading to the considerable increase in weight, as reported by Aremu A in 2018[15]. In this study it is found that oral administration of 500 mg/kg Moringa extract for 28 days significantly increased weight when compared to the negative control. These findings are consistent with Jameel H(2018) and Mohlala K (2023) demonstration of a significant weight gain following oral administration of the extract in an arthritic rat model for 18 days.[14-18] According to A. Aremua's 2018 research, rats infected with trypanosome brucei within 14 days gained more weight when given MO leaves.[15] Diana Meirelesi's review article from 2020. suggested consuming capsules and tea made from moringa leaves to enhance absorption and digestion, which may assist with the healing process of a variety of inflammatory disorders.[17]

There was a significant difference in the mean cartilage damage depth values between the vitamin D treatment group C, the negative control group B, and the control group A. The average difference between the control and Moringa-treated groups was insignificant. Iffah Nadhira in 2019 and Jitender Malik in 2024 found that the negative control group lost more cartilage as inflammation advanced to calcified cartilage and subchondral bone.[19-20].

The present study employed oral administration of vitamin D and Moringa extract at doses of 4000IU/kg and 500mg/kg, respectively, to reduce the extent of cartilage breakdown; Moringa extract excelling vitamin D. Mohamed E. Mansour in 2023 stated that Moringa utilization reduced the cartilage deterioration and synovial hyperplasia in diseased rat joint21. In 2012, researchers discovered that oral vitamin D supplementation at a dose of 4 IU/kg reduced cartilage deterioration in an arthritic rat model. Sanjay Kumar in 2023 reflected the effects of vitamin D in various inflammatory disorders and demonstrated that chronic inflammatory diseases tend to have lower vitamin D status, underlining the role of vitamin D in lowering inflammation.[3] Ana Divjak in 2023 concluded that supplementing with vitamin D3 could be a potential treatment for osteoarthritis in the knee.[22]

In 2016, Nishat Fatima discovered that Moringa extract can slow cartilage degeneration in arthritic rat models. Similarly, Zhengling Zhuang proved the anti-inflammatory properties of kaempferol, a powerful

flavonoid present in Moringa leaves, which inhibited NF-kB activation in rat chondrocyte cells stimulated with interleukin-1b.[23] Diana Mireles in a review study identified the antioxidant and anti-inflammatory effects of vitamin A and C in Moringa oleifera leaves.

However, this investigation has limitations. Surgically induced OA models of the knee joint are ideal for short-term investigations, but they are expensive and require more expertise and apparatus. The study failed to look at the changes at the molecular level. Further research could explore the combined benefits of vitamin D and Moringa extract on articular cartilage in OA.

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CONCLUSION

In conclusion, Moringa oleifera was discovered to be a potent anti-inflammatory agent than vitamin D in repairing histomorphological alteration in articular cartilage in arthritic rat model. This study also investigated nutritional and therapeutic characteristics of Moringa oleifera leaves in an arthritic rat model.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AS & SA: Data acquisition, data analysis, critical review, approval of the final version to be published.

TQ & IZ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

KA & N: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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