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# In vivo investigation of the ameliorating effect of copper albumin complex on chondroitin sulfate in monosodium iodoacetate -induced knee osteoarthritis in rats

**Doaa A. Elnakip[1], Nashwa A.M. Mostafa[2], Asmaa A. Metwally[3*], Reham I. El-Mahdy[4], Ahmed Y. Nassar[4], Obeid Shanab[1], Khaled Ghareeb[5], Ahmed Abdeen[6,7], Zainab M. Maher[8], Mohamed A. A. Mahdy[9], Aya Sh. Metwally[10], Mohammed Salah[1]**

[1]Department of Biochemistry, Faculty of Veterinary Medicine, South Valley University, Qena 83523, [2]Department of histology and cell biology, Faculty of Medicine, Assiut University, Assiut, [3]Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, [4]Department of Medical Biochemistry and Molecular biology, Faculty of Medicine, Assiut University, [5]Department of Animal and poultry behavior and management, Faculty of Veterinary Medicine, South Valley University, Qena 83523, [6]Center of Excellence for Screening of Environmental Contaminants (CESEC), Faculty of Veterinary Medicine, Benha University, Toukh, [7]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh, [8]Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, [9]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, [10]Department of Pharmacology, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt.

## Abstract

Osteoarthritis (OA) is a condition that manifests as cartilage deterioration and subchondral bone sclerosis in the joint tissues. The weight-bearing joint is most severely impacted by OA. According to some research, consuming foods high in copper albumin complex (cu-albumin complex) can help with OA-related joint degeneration and pain relief. The current study's objective to determine how oral administration of the cu-albumin complex as an anti-inflammatory medication affected the development of rat knee osteoarthritis (KOA). Fifty adult albino rats were divided into three groups: negative control untreated (n= 10, no KOA induction); positive untreated control (n= 20, KOA induction); and treated group (n= 20, KOA induction with administration of cu-albumin complex). According to the severity of the clinical symptoms, treated and untreated arthritic groups were equally divided into mild and severe groups (n=10). Monosodium iodoacetate (MIA) was used as intra-articular injection for osteoarthritis induction. Rats were euthanized after a month of the beginning of the experiment, and the joints were examined histopathologically and immunohistochemically. It was indicated that the treatment was effective in reducing KOA severity and in improvement of chondroitin sulfate of the affected cartilages. In conclusion, the structure of the chondroitin sulphate in the knee joint cartilages of KOA-affected rats was modified by the cu-albumin complex.

**Keywords:**

Chondroitin sulfate, Copper-albumin complex, Mankin scores, Osteoarthritis.

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*Corresponding Author: Asmaa A. Metwally E-mail: asmaaabdelsalam104@yahoo.com

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## Introduction

Osteoarthritis (OA) is a crippling condition that mainly affects the body's weight-bearing joints, such as the hips and knees (Alluri et al., 2020). In addition to synovitis, subchondral bone sclerosis, and osteophyte formation, it is characterized by increasing cartilage degradation (Zhang et al., 2019; Fonsi et al., 2020) and loss of type II collagen and glycosaminoglycans (GAGs), which are typically present in the healthy joint (Reid, 2013).

A complex network of interacting mechanical, biological, biochemical, molecular and enzymatic feedback loops leads to OA. The breakdown of joint tissue due to cells' inability to maintain a homeostatic balance between matrix synthesis and degradation is the final common process. Progressive joint tissue lesions result from the disease's degradative phase finally surpassing its anabolic counterpart as the condition worsens. This seems to happen when the physiological balance between the extracellular matrix's synthesis and degradation favors catabolism (Martel-Pelletier and Pelletier, 2010).

The loss of the chondrocyte matrix results in the degradation of the joint cartilage, which is a feature of OA, despite the normal bone remodeling enhanced by osteoclast and osteoblast cells under the direction of para-thyroid hormone. But synovitis also contributes to the establishment of the pathology (Martel-Pelletier and Pelletier, 2010; Jaleel et al., 2020).

Age, being overweight or obese, joint injury or instability, gender, heredity, metabolic/endocrine diseases like diabetes, and crystal deposition disorders like gout are the main risk factors for developing OA (Bortoluzzi, Furini and Scirè, 2018).

Radiography images of OA show synovial membrane thickening and articular surface erosions in degenerative arthritis. It can be used effectively in the detection of degenerative arthritis lesions (Razek and El-Basyouni, 2016; Jaleel et al., 2020).

Extracellular matrix (ECM) is made up primarily of chondroitin sulfate (CS), a naturally occurring bioactive macromolecule. Chondroitin sulfate is found in a wide variety of organisms and has drawn considerable interest because of its potent bioactivities (Yang et al., 2020).

The synovial fluid's viscoelastic component and chondroitin sulfate, a crucial part of the cartilage matrix (Fonsi et al., 2020). The treatments currently available for OA do not provide a cure and simply temporarily reduce symptoms (Zhang et al., 2019).

The study aimed to evaluate the anti-inflammatory effect of copper albumin complex for reduction of the joint cartilage degradation and increasing chondroitin sulfate in the rats' KOA model.

## Materials and methods

### 1. Ethical Approval

Animal Research Ethics Committee in The Faculty of Veterinary Medicine, South Valley University, Qena, Egypt approved all experimental protocols (VM-2022-0032).

### 2. Drugs and chemical

Mono-iodoacetate (MIA) was purchased from Sigma Aldrich (St. Louis, MO, USA); Rat proteoglycan Eliza kit was purchased from SinoGeneclon CO., Ltd. (Hangzhou, China); Cu-albumin complex was obtained from Prof. Dr. Ahmed Yassein Nassar, professor of biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt, as a patent cooperation treaty (PCT) in the International Bureau of

World Intellectual Property Organization (WIPO), Geneva, Switzerland.

### 3. Animals

Adult male albino rats (n=50), of 130 to 150 g body weight and of 12 weeks age were procured from the animal house of the Faculty of Medicine at Assiut University in Assiut Governorate. Rats were kept in groups in a clean, ventilated room with a controlled humidity and temperature (50±5% and 24±2℃) and a 12-hour cycle of light and dark. Feed and water were freely available. Intra-articular injection of MIA (3 mg in 50 µl sterile saline) was used to induce experimental osteoarthritis in rats after 10-days adaptation period (Guingamp et al., 1997; Udo et al., 2016; Xu et al., 2020). The hair on the right knee was shaved using an electric razor. The shaving was performed after anesthetized the rats by ethyl acetate anesthesia. Following cleaning the area, an incision was made in the center of the knee to expose the patellar ligament. The injection was administered into the area below the patella after identifying the patellar ligament with the right leg flexed 90˚ at the knee (Takahashi et al., 2018).

### 4. Experimental groups

Following one week after KOA induction, rats were randomly divided into five groups, each consisting of ten rats, and the following assignments were made:

**A-** Ten normal healthy rats kept as a **negative control group**.

**B-** Twenty non-treated rats kept as a **positive control**, where rats in this group were not given any treatment following the induction of osteoarthritis, and they were divided equally into mild and severe non-treated groups (10 rats each) based on the severity of the clinical signs (pain, swelling, and lameness).

**C- Twenty treated rats' group**, where the rats were divided into **mild** and **severe** treated groups (10 rats each) based on the severity of their symptoms. After dissolving 817µg/kg of copper albumin complex in water, the rats were given copper albumin complex orally once daily for a month at a dose of 1 ml/kg B.W (Taha et al., 2022).

### 5. Animal assessment and Samples collection

The swelling of the right knee joint was evaluated once weekly using a digital caliper in millimeters (Vogel), and the accelerated rotarod apparatus (Ugo Basile, Varese, Italy, Model 7750) was used to determine the joint mobility, motor function, and pain (Vonsy, Ghandehari and Dickenson, 2009).

### 6. Knee joint histopathological analysis (right femorotibial joint)

The right knees from all groups of rats were taken, cleaned with saline solution, and stored for a week in a solution of 10% ethylene diamine tetra-acetic acid (EDTA) for decalcification. This was done after the rats were euthanized via cervical decapitation at the end of the experiment. Joints were fixed in 4% neutral buffered formalin (NBF) for 48 hours after decalcification. All samples were prepared using the standard histopathological method. The sections (5µm thick) were cut with a Microtome (Leica RM2235, Leica Bio-systems) and staining them with the H&E and Crossman's trichrome stains and examined under the light microscope (Suvarna, Layton and Bancroft, 2018). All slides were graded using the modified Mankin score's parameters (McNulty et al., 2012; Cui et al., 2015).

### 7. Statistical analysis

Using SPSS, the acquired data were represented as means and standard deviation (Statistics package version 17.0 SPSS Chicago, IL, USA). Quantitative differences between values were analyzed using one-way analysis of variance (ANOVA), and multiple intergroup

comparisons were analyzed using Dunnett post hoc tests. A paired sample t-test was used to compare the two dependent groups. Statistical significance was defined as a P-value of <0.05.

## Results

### 1. Histopathological examination

### 1.1. H and E stain:

The control group's knee joints displayed normal joint histology, including a smooth, undamaged surface and normally organized chondrocytes of the articular cartilage. A clear intact tidemark that showed as a basophilic line between the two sections divided the articular cartilage's non-calcified and calcified cartilage regions. Three zones of chondrocytes were present in the non-calcified area: the superficial (tangential), transitional (intermediate), and radial (deep) zones. Small flat chondrocytes were organized parallel to the articular surface in the superficial zone. Round, oval, or triangular chondrocytes were organized in columns perpendicular to the surface in the transitional and radial zones. The chondrocytes were found inside their lacunae either singly or in groups forming cell nests. They have pale basophilic cytoplasm with central spherical nuclei. Round chondrocytes were dispersed across the calcified region and were found in their lacunae (Fig. 1).

Additionally, compared to the control group, the histology of the mild osteoarthritis group revealed several histological alterations. The disruption of the chondrocytes' parallel arrangement, the irregular notched surface, and the apparent reduction in cartilage thickness, these changes were of various severities. In addition, the tidemark was faint and asymmetrical, and certain chondrocytes seemed shrunken with pyknotic nuclei, disorganized and few, and loss of chondrocytes in some locations (Fig. 2A).

On the other hand, the articular cartilage deterioration was seen in severe osteoarthritis. Additionally, empty, deteriorated, and disorganized chondrocytes without a tidemark were seen (Fig. 2B). Contrary to the osteoarthritis group, the treated groups showed a better histological appearance. Except for few chondrocytes that had decreased, the mildly treated group displayed less degenerative alterations and appeared practically normal (Fig. 2C). The severely treated group displayed enhanced cellularity with reduced chondrocyte degeneration and subtle tidemarks (Fig. 2D).

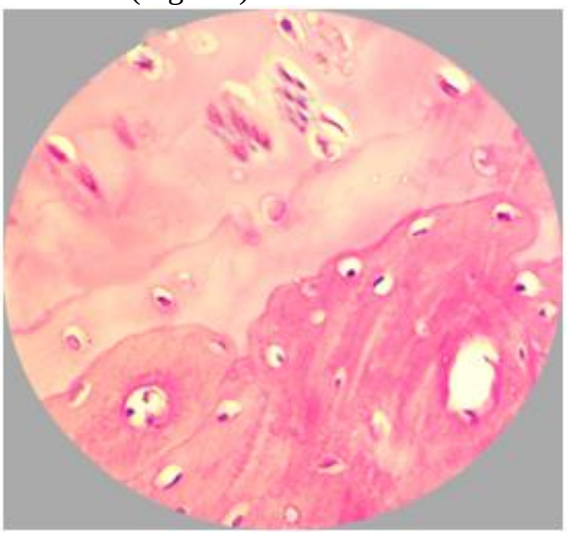

**Fig. 1.** H and E–stained section of the normal knee joint with regular smooth articular surface and well-organized chondrocytes x400

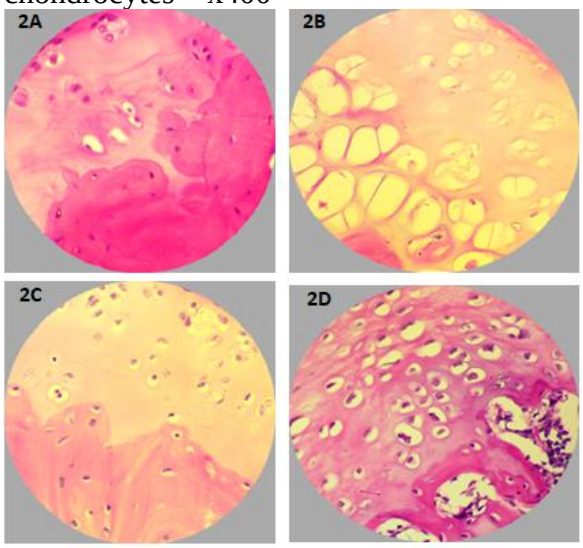

**Fig. 2. Showing H and E-stained sections of treated KOA rats. A.** Mild KOA, showing irregular articular surface with disorganized chondrocytes x400. **B.** Severe KOA, showing marked degeneration of articular cartilage with disarranged and degenerated empty chondrocytes x400. **C.** Mild treated KOA showing less articular cartilage degeneration with few degenerated chondrocytes x400. **D.** Severe treated KOA, showing increased cellularity with decrease degenerated chondrocytes.

### 1.2. Immunohistochemistry of chondroitin sulfate:

Chondroitin sulfate immunohistochemistry in the control group revealed extremely positive immunostaining in the cartilage matrix (Fig. 3A). While immunostaining decreased in the group with mild osteoarthritis (Fig. 3B). Additionally, the cartilage matrix immunostaining for chondroitin sulfate in the group with severe osteoarthritis showed a notable decrease (Fig. 3C). On the other hand, the mildly treated group's immunostaining was found to have significantly increased (Fig. 3D). While there was a little rise in immunostaining in the severe treated (Fig. 3E).

### 2.The mean area percentage of chondroitin sulfate immunostaining:

The mean area percentages of chondroitin sulphate in the mild and severe untreated osteoarthritis group dramatically reduced as compared to the control. The mean area percentage of chondroitin sulfate in both treated groups was significantly lower than the control group. There was a highly significant increase in the mild and severe treated groups compared to the mild and severe osteoarthritis groups (Fig. 4).

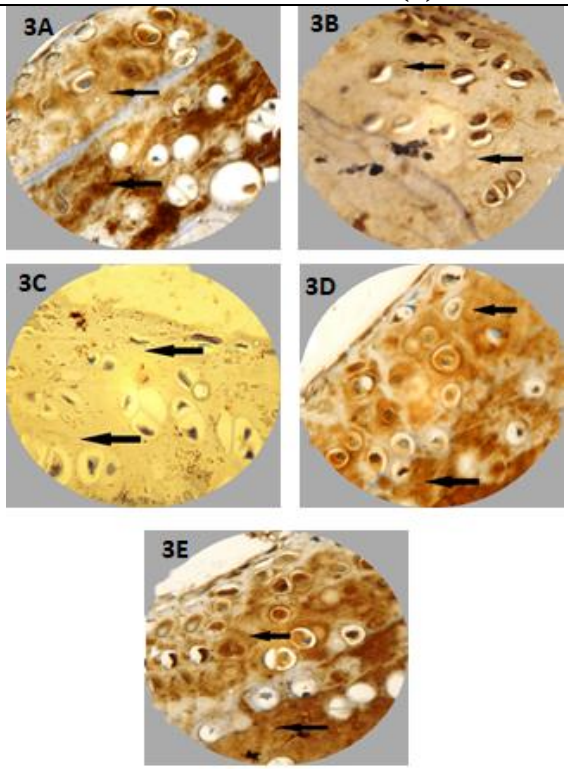


**Fig. 3. Immunohistochemistry of chondroitin sulfate in: A. Control group:** showing highly positive immunostaining in the cartilage matrix (arrow), **(B-C). Mild osteoarthritis group:** showing decreases in the immunostaining in the matrix (arrow), **D. Mild treated group** showing a marked increase in the immunostaining (arrow), **E. Severe treated group**, showing a moderate increase in the immunostaining (arrow). x1000.

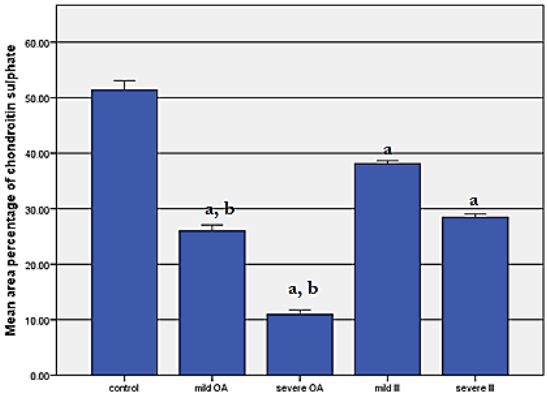


**Fig. 4. Histogram represents the mean area percentage of chondroitin sulfate immunostaining in all experimental groups.** ([a]); significant changes when compared with the control group when $p$ is $\leq 0.05$. ([b]); significant changes when compared with mild and severe treatment groups when $p$ is $\leq 0.05$

**3. Histological scoring (Mankin score):**

The control group's Mankin score was 0. When compared to the control group, the stained sections from the osteoarthritis group revealed highly significant articular cartilage damage, with scores of 7.40±1.02 mm for mild osteoarthritis and 11.7±1.3 for severe osteoarthritis (irregular notched surface, hypocellularity, severe reduction in the matrix staining intensity, and invisible tidemark) (Table 1). With a score of 4.20±1.3 in the mild and 6.5±0.6 in the severe treated groups , the stained sections of the treated osteoarthritis group showed highly significant less degenerative changes in the articular cartilage as compared to the osteoarthritis group, indicating that the treated groups was associated with better preservation of articular cartilages (Table 2).

**Table 1: Manikin's total score (1-14) is the sum of the scores for cartilage structure, cellular abnormalities, tideline, and toluidine blue staining.** A total score of 14 indicates extensive cartilage destruction, whereas a score of 0 indicates normal cartilage.

| Cartilage structure | Score |
|---|---|
| Normal | **0** |
| Surface irregularities | **1** |
| Pannus and surface irregularities | **2** |
| Clefts to a transitional zone | **3** |
| Clefts to radial zone | **4** |
| Clefts to calcified zone | **5** |
| Complete disorganization | **6** |
| **Cellularity** | **Score** |
| Normal | **0** |
| Diffuse hyper-cellularity | **1** |
| Cloning | **2** |
| Hypo-cellularity | **3** |
| **Toluidine blue staining** | **Score** |
| Normal | **0** |
| Slight reduction | **1** |
| Moderate reduction | **2** |
| Severe reduction | **3** |
| Absent | **4** |
| **Tidemark integrity** | **Score** |
| Intact | **0** |
| Crossed by blood vessels | **1** |

**Table 2: The total histological lesions score (Mankin score) of knee cartilage** in the control, moderate untreated osteo-arthritis (OA), severe untreated OA, mild treated OA, and severely treated OA animal groups. Data expressed as mean ± SD. $P_1$ vs. control group; $P_2$ vs. Mild OA group; $P_3$ vs. severe OA group, $P_4$ vs. mild treated OA group, and NS; non-significant. Statistical analyses were performed by one-way analysis of variance with Tukey's post hoc test (P <0.001).

| Group | Mean ± SD | P1 | P2 | P3 | P4 |
|---|---|---|---|---|---|
| **Control** | 0.00±0.00 | | | | |
| **Mild osteo-arthritis (OA)** | 7.4±1.02 | <0.001 | | | |
| **Severe OA** | 11.7±1.30 | <0.001 | <0.001 | | |
| **Mild treated OA** | 4.2±1.30 | <0.001 | <0.001 | NS | |
| **Severe treated OA** | 6.5±0.60 | <0.01 | <0.01 | <0.001 | <0.001 |

**Discussion**

Osteoarthritis (OA) is a chronic, incapacitating originating from a confluence of risk factors, including ageing, heredity, trauma, misaligned knees, higher biomechanical loading of joints due to obesity, increased bone density, and an imbalance in physiological processes. These factors lead to catabolic cascades at the molecular level (Martel-Pelletier and Pelletier, 2010). OA also results in impairments and lowers quality of life (Martel-Pelletier, Barr and Cicuttini, 2016; Bowden et al., 2020). Compared to OA in the other joints, KOA is more frequently linked to disability (Li et al., 2020).

Articular cartilage is a connective tissue with a low capacity for self-repair. It is avascular, hypocellular, and hyaline. Four different zones make up the mature tissue; they are referred to as (a) superficial/tangential; (b) intermediate/mid; (c) deep; and (d) calcified. Each zone's cellular morphology, extracellular matrix (ECM) makeup, and collagen fiber organization vary significantly across the tissue depth, and together they play a role in the articular cartilage's overall ability to resist biomechanical load (Poole et al., 2001).

The extracellular matrix of the articular cartilage, including chondroitin sulfate as one of the major components, is crucial for the normal functioning of the joints and plays a significant role in skeletal development (Watanabe, Yamada and Kimata, 1998).

Chondroitin sulfate inhibits chondral degeneration by inhibiting hydrolytic and proteolytic enzymes, lessening collagenase activity, attenuating oxidative events, and thereby reducing the progression of osteoarthritis (Bottegoni et al., 2014; Morita et al., 2018; Yamada et al., 2022).

Pelletier, Martel-Pelletier and Abramson (2001) have found that osteoarthritic patients exhibited pathological alterations in the afflicted joint, such as inflammatory synovitis, pannus-like synovial tissue (Shibakawa et al., 2003), cartilage erosions, late osteophytosis, subchondral bone remodeling, chondrocytic apoptosis, and alterations in proteoglycan content and collagen composition (Li et al., 2020).

Numerous OA studies employ MIA-induced OA models. Depending on the purpose of the study, different dosages are used in the MIA-induced KOA model. Therefore, the amount of MIA should be modified in accordance with the investigated osteoarthritis model (Yoh et al., 2022).

The histological changes in MIA-induced KOA mice have been shown to be dose-dependent (Udo et al., 2016). Following a localized acute inflammation brought on by MIA, cartilage degradation occurs (Marker and Pomonis, 2012).

Inflammation is linked to the progression of OA (Goldring and Otero, 2011). Therefore, in experiment carried out by Yoh et al. (2022) they indicated that the 1.0-mg, 2.0-mg, and 4.0-mg MIA groups all have OA changes after one week of injection, and that the severity of these changes increased as the MIA dose increased.

Several doses (0.1, 0.25, 0.5, and 1.0 mg) of MIA were injected into the knee joint, and the histological examination was performed three weeks after induction. At 0.1 mg of MIA, a low dose, mild cartilage damage was seen; at 1 mg, a high dose, severe damage was noticed. For three weeks, cartilage damage has been observed at the MIA dose of 0.25 mg (Janusz et al., 2001).

One of the most urgent issues in modern medicine today is how to treat osteoarthritis (OA). This is due to the OA is a very common pathology with severe symptoms and consequences that can lead to disability and there is a lack of data on the effectiveness of various treatments and medications (Materkowski, 2021; Mao et al., 2021).

However, there are analgesic and anti-inflammatory medications available, including corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) (Liu et al., 2017), instead of offering a cure, these traditional medicines focus more on reducing symptoms. Joint replacement surgery is also the only available treatment option for patients with end-stage OA. In despite the fact that the procedure itself has

drawbacks such high related costs, perioperative complications, and infections (Gunaratne et al., 2017).

Copper is an important trace element, which is crucial for keeping the physiological equilibrium and antibiosis in the body (Skaar and Raffatellu, 2015; Bost et al., 2016).

By comparing the results between the experimental rat groups in the current investigation, it was shown that rats with mild and severe osteoarthritis had degenerative-dystrophic changes in their knee joints. Rats in the severe osteoarthritis group showed the most marked severity of degenerative alterations. As a result, it is possible to prove that oral administration of the copper-albumen complex exhibited a reduced intensity of damaging alterations in the cartilaginous surface of the knee joint. As the treated mild and sever osteoarthritic group showed increase in chondroitin sulfate in comparison with non-treated mild and sever osteoarthritic groups. Djoko et al. (2015) confirmed that, both cellular and humoral immunity depend on copper. Additionally, Tapiero, Townsend and Tew (2003) showed that, the acute phase reactivity to inflammation, infection, and other disorders clearly increases copper metabolism. Also, Fraga, (2005); Lin et al. (2019), and Medeiros, (2016) stated that, copper plays a key role in the production of numerous cellular enzymes, including the copper-containing superoxide dismutase, cytochrome oxidase, and lysyl oxidase, in healthy tissues, especially cartilage, in addition to its positive effect on immune response

In animal models, histopathology, particularly of cartilage, has been the main outcome measure utilized to evaluate OA and treatment response. The majority of the histopathological scoring methods in use are based on those first described by Mankin et al. (1971) which rate the integrity of tidemarks, cellularity, cartilage structure, and proteoglycan staining (Mankin et al., 1971; Little et al., 2010).

The proteoglycan aggrecan (PG) of articular cartilage was graded using the Mankin technique to identify structural alterations. The results showed that by comparing the control group, osteoarthritis group revealed highly significant articular cartilage damage, with Mankin scores of 7.40±1.02 mm for mild osteoarthritis and 11.7±1.3 mm for severe osteoarthritis (irregular notched surface, hypocellularity, severe reduction in the matrix staining intensity, and invisible tidemark). With a score of 4.20±1.3 mm in the mild and 6.5±0.6 mm in the severe treated groups, the stained sections of the treated osteoarthritis group showed highly significant less degenerative changes in the articular cartilage as compared to the osteoarthritis group. These indicating that the treated groups was associated with better preservation of articular cartilages (Lark et al., 1997).

## Conclusion

From the previous mentioned results, it can be concluded that treatment of KOA with oral supplementation of copper albumin complex can improve the joint cartilage health and decrease the deteriorative effect of osteoarthritis in the cartilage structure and improve the chondroitin sulfate in the of Knee joint cartilages of rats.

## Authors' contributions

DAE., MSA., AYN and OS conceived and designed the study. OS and DAE conducted the experiment and collected the data. NAMM., ZMM and RIE performed histopathological and immunohistochemistry studies. ASM., MAAM., KG and AA organized, analyzed and interpreted the data. ZMM and AAM wrote the paper and revised the final draft.

All authors have read and agreed to the published version of the manuscript.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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