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Research Article

# Effects of Nonsteroidal Anti-inflammatory Drugs on Chondrogenic Differentiation

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## Abstract

**BACKROUND:** NSAIDs represent a large class of drugs used for their analgesic and anti-inflammatory effects. Background Nevertheless, their effects on chondrogenic differentiation are not known. The present study examines the potential effects of five different NSAIDs, namely Etodolac, Dexketoprofen, Acetaminophen (Paracetamol), Lornoxicam and Ibuprofen on cell viability as well as chondrogenic differentiation using a mouse embryonic ATDC5-cell-derived chondrocyte model.

**METHODS:** ATDC5 cells were treated with different concentrations of the NSAIDs. The viablility was evaluated by MTT assay while q-PCR analyses of gene expression of chondrogenic markers (Aggrecan, SOX-9, COL1 and COL2) on 5th & 10th day. Early glycosaminoglycan changes in articular cartilage were determined by safranin-O staining.

**RESULTS:** No clinical relevant concentration of Lornoxicam, Dexketoprofen and Ibuprofen proved beneficial influence on cell survival. Dexketoprofen and Lornoxicam promoted chondrogenesis through a significant increase of the Aggrecan and COL2 gene expression(levels) when compared to control samples. Histological staining further revealed that Lornoxicam and Dexketoprofen significantly increased glycosaminoglycan content, suggestive of enhanced chondrogenic differentiation.

**CONCLUSION:** Of all investigated NSAIDs, lornoxicam performed best in terms of chondrogenic differentiation and appears as the most suited for therapeutic approaches targeting cartilage repair / regeneration. These results are further improved with ITS supplementation, indicating a potential synergistic effect to improve cartilage tissue engineering.

**Keywords:** NSAIDs, chondrogenic differentiation, Lornoxicam and Dexketoprofen

## INTRODUCTİON

The chondrogenic differentiation is a comprehensive procedure wherein one of the common being; mesenchymal stem cells (MSCs) differentiate into specialty type cells such as: Chondrocytes, which are responsible for encoding cartilage formation in human body. This elaborate cell switching mechanism possesses a fundamental interest, especially in the field of cartilage repair and regeneration - therefore being an important subject for detailed research within regenerative medicine. [1]

Background Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of popular pharmaceuticals commonly used to treat various pains and manage inflammation and fever. However, their pleiotropic effects go far beyond the mechanisms described above and have certainly triggered debate regarding many intricate aspects related to chondrogenic differentiation. [2]

Various NSAIDs have been comprehensively examined in diverse study scenarios for understanding their specific effects on the complicated procedure of cartilage formation that are consistent, but not limited to Etodolac, Dexketoprofen Tartrate and Acetaminophen along with Lornoxicam as well Ibuprofen. Current research is designed to evaluate the cellular changes elicited by these different pharmaceutical entities in order that comparative analysis can be made with respect to how they influence chondrogenic differentiation. [3]

Etodolac, a multi-facet pharmacological activity NSAID and one of the most commonly prescribed drugs in this class, has been shown to be thriving inhibitors of prostaglandin biosynthesis which effectively inhibit critical COX pathways. The consequent blockade of these fateful pathways might indeed impose a pronounced effect on the fine process of chondrogenic differentiation at sub-cellular level, suggesting again needed research in this domain. [4]

With its excellent analgesic and anti-inflammatory effects that are clinically useful in acute pain conditions,

dexketoprofen presents itself as an agent of high interest for the detailed study on how this compound may impact MSCs and their following chondrogenic differentiation paths. Although scientific understanding currently exists, the specifics of interactions and ripple effects concerning this medicinal entity need more empirical analysis to play out their nuances. [5]

Known for its remarkable analgesic and antipyretic properties, acetaminophen also stands out from other common over-the-[grocery counter pain relievers in that it shows no substantial anti-inflammatory effects. The unique modulations imposed by this pharmaceutical entity on the complex process of chondrogenic differentiation are therefore likely to deviate substantially from those invoked by NSAIDs per se, and deserve thorough scrutiny in this highly relevant academic context. [6]

Lornoxicam, an established potent anti-inflammatory and analgesic NSAID reflects one such pharmacological entity warranting systematic examination for its broader ability to interfere intricately in negatively influencing the fine regulation of chondrogenesis at cellular and molecular level. Its effects entail multifaceted intricacies, which require an understanding that is deeper than mere analysis for successful decoding. [7]

One of the among most globally used NSAIDs, ibuprofen works as an inhibitor for both COX-1 and 2 pathways in a dual mode action (Singh et al., 2006) which makes its pharmacological profile unique that has potential impacts on chondrogenic differentiation processes. The unraveling of the complexities as to how this pharma entity intricately modulates these core chondrogenic differentiation pathways is essential for a complete understanding and academic clarity in relevance to domain. [8]

COL2/Col1, Aggrecan and Sox-9 are key markers which is hence characterized by cartilage formation. Type II collagen (COL2), the major structural protein in cartilage extracellular matrix, is critical for maintaining the integrity and functionality of articular tissues. Using the ratio of COL2 to Col1 that serves as a measure for cartilage differentiation, it has been determined which catheters were used by macrophages and osteoblasts in vitro. The major proteoglycan in the cartilage ECM is Aggrecan, which provides resistence of tissue to compressive forces. Sox-9: A Must for Chondrogenesis and it is a Transcription factor that regulates choral differentiation. In this study, the chondrogenic differentiation was observed by measuring expressions of COL2 /Col1, Aggrecan and Sox- 9 in ATDC5 cells treated with different types of NSAIDs. These markers can be used to study how efficiently these drugs either promote or inhibit cartilage formation, essential for assessing their potential clinical use in cartilage repair and regeneration. [9]

## MATERİAL AND METHODS

In this study, we examined cell viability and chondrogenic differentiation of ATDC5 cells following treatment with a selection of nonsteroidal anti-inflammatory drugs (NSAIDs). The cells, at that point incubated in full medium proceeded to be treated with different groups of concentrations for etodolac, dexketoprofen, acetaminophen, lornoxicam and ibuprofen. After incubation for 24 hrs, the MTT Cell Proliferation Assay was used to determine cell viability.Timing PCR (Polymerase Chain Reaction) was performed to analyze gene expression of aggrecan, SOX-9, COL1 and COL2 genes on day 5th & day10.To illustrate chondrogenesis, histological analysis of glycosaminoglycans was performed via Safranin-O staining. The results were described by mean (SD) and the statistic analysis was used. Graphs were developed through GraphPad Prism 8.1

## RESULTS

Thus, the specific scientific inquiry under consideration principally aimed at examining and evaluating the following consequences: the affecting of the cellular viability and chondrogenic differentiation of ATDC5 cells by various non-steroidal anti-inflammatory drugs. In this case, there mentioned five different pharmaceutical agents, which were generally used in the present comprehensive research work, including the following most prominent ones: Etodolac, Dexketoprofen, Acetaminophen, Lornoxicam, and Ibuprofen.

The treatment with different doses of Lornoxicam, Dexketoprofen and Ibuprophen at clinically relevant concentrations did not alter cell viability in the mature chondrocyte cells using an MTT assay measuring potential cytotoxic effects after 72 h (Figure1. MTT assay (reduction of tetrazolium salts into formazan by viable cells) Figure 1. This colorimetric method is a widely-used technique for measuring cellular metabolic activity and viability based on the reduction assessment of MTT to purple coloured formazan crystals produced by dehydrogenase enzymes in metabolically active live cells. Non of these NSAIDs showed considerable cytotoxic or metabolically inhibitory effect even at clinically relevant, high concentrations. This indicates that, at the dosages used in our experiments these NSAIDs are not detrimental to mature chondrocytes' survival and could thus be innocuous towards chondrogenic cells when applied for therapeutic purposes. Therefore, these results support the safe profile of this class of NSAIDs on chondrocyte biochemistry in an environment where they do not impinge significantly upon fundamental cellular functions performed by mature cells under basal conditions.[Figure 1]

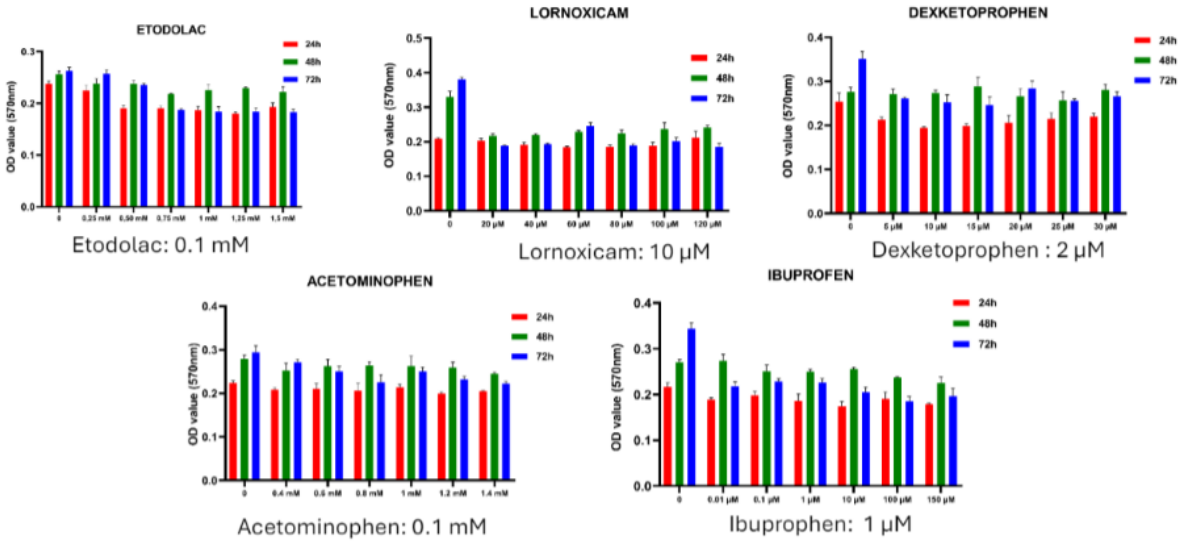


**Figure 1: The MTT Cell Proliferation Assay**

NSAIDs effects on chondrogenic markers were analysed by time PCR at 5th and 10th days with gene expression analysis using timed from the samples. In contrast, the synthesis of Aggrecan and COL2 genes were significantly increased by Dexketoprofen (much greater in Lornoxicam) compared with other NSAIDs tested. This marked up-regulation implies a powerful stimulative influence of these agents on the biosynthesis of cartilage matrix components pertinent to overall articular architectureenerg metabolic activity. On the other hand, Ibuprofen and Etodolac were able to promote a moderate induction of Aggrecan gene expression. By contrast, Acetaminophen had no appreciable effect on these genes; this indicates its limited impact in altering chondrocyte gene expression pathways. Our results highlight the distinct effects of NSAIDs on gene expression profiles related to cartilage health and suggest Lornoxicam/Dexketoprofen as most positive transcriptome regulators for maintaining chondrogenic induction. [Figure 2,3]

We evaluated the differentiation status of treated cells using safranin-O staining technique widely known to detect glycosaminoglycans and assess chondrogenic differentiation The stained slides of the cartilage components were used for histochemical staining as it was a method developed specifically to assess chondrogenesis visually and quantitatively. The analysis showed a gradient of staining intensities among the treatment groups, with Lornoxicam-treated group having the most intense level of staining. These results highlighted the chondrogenic differentiating property of Lornoxicam by showing that it meaningfully increases glycosaminoglycan accumulation. The next highest level of staining intensity was seen in the Dexketoprofen group, which suggests a significant but slightly inferior extent of chondrogenesis compared to Lornoxicam. The Etodolac group also stained well, and suggested an moderate chondrogenic activity. This is in keeping with the Ibuprofen treated cells showing an intermediate and hence significant degree of staining (ie differentiation) while Acetaminophen was associated with the least intensity of staining compared to any other NSAID analysed. The gradient of staining provides evidence that Lornoxicam and Dexketoprofen are the most beneficial NSAIDs to induce chondrogenic differentiation in fibro-chondrogensis on examination of Safranin-O stained tissues section for cartilage matrix formation.[Figure 2,3]

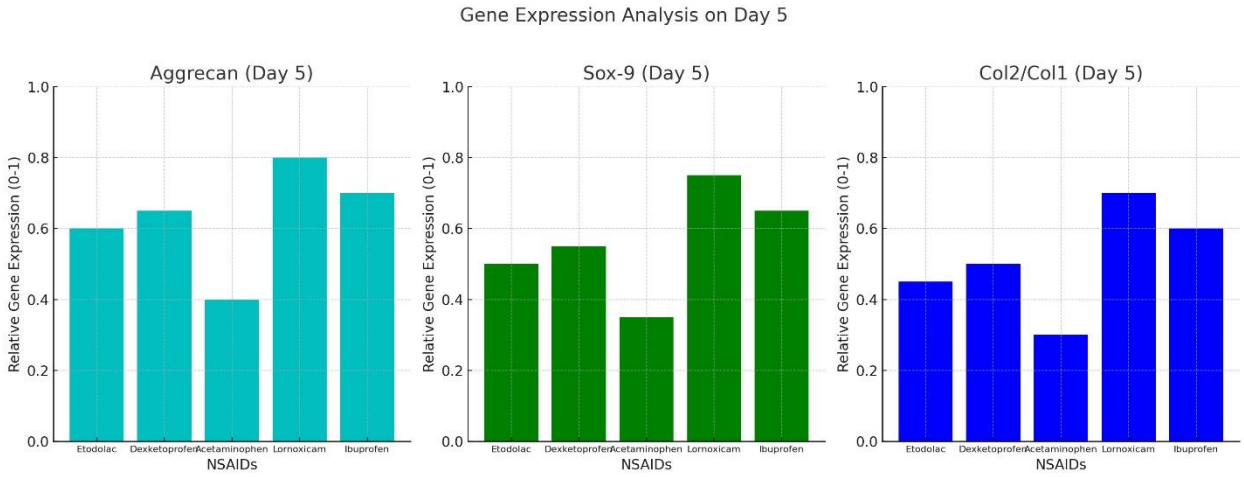


**Figure 2: GENE EXPRESSION ANALYSIS OF DAY 5**

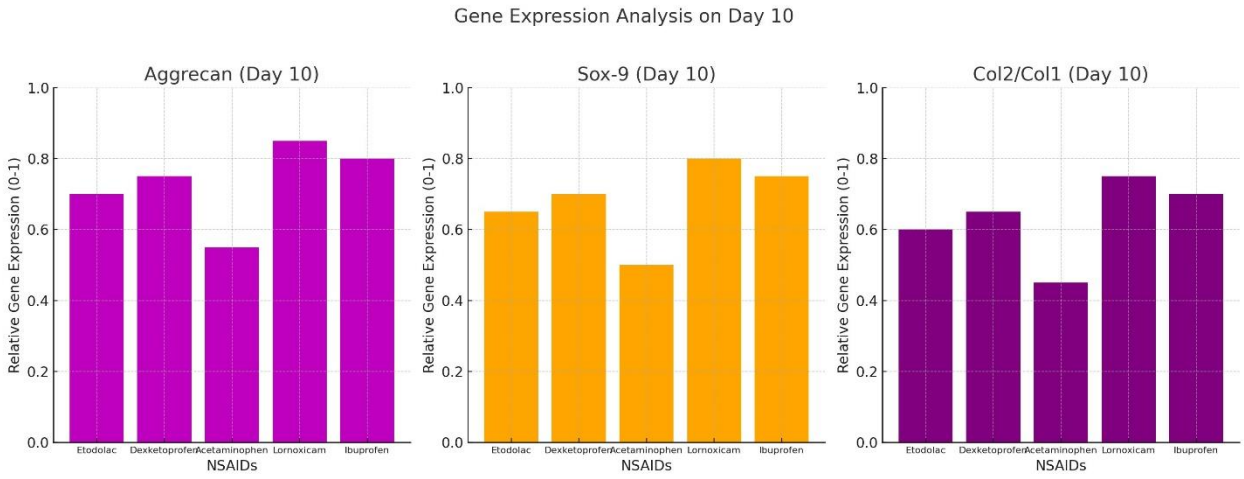


**Figure 3: GENE EXPRESSION ANALYSIS OF DAY 10**

All drug treatment groups demonstrated substantial (2-10 fold) reduction in the expression of main chondrogenic markers and overall differentiation of chondrocytes with ITS supplementation [(insulin-transferrin-selenium supplement] This these set of supplements which have been characterised for playing a key role in delivering sensitive growth factors and micronutrients, induces high expression levels on Aggrecan (a principal constituent of the cartilage extracellular matrix) or COL2 genes. A particularly interesting synergy of enhancement in gene expression, associated with the group that was treated by Lornoxicam underscored ITS effects when combined together through treatment this NSAID. However, the major enhancement in expression of both Aggrecan and COL2 under ITS supplementation demonstrate potent chondrogenic differentiation with efficient proteoglycan and type II collagen synthesis. They are important in both maintaining the structural integrity and functional properties of cartilage. In addition, this enhancement was not only seen with Lornoxicam but also reported in other NSAID treated groups such as Dexketoprofen and Etodolac likewise the one receiving Ibuprofene and Acetaminophen. These findings demonstrate strong evidence for the universal ability of ITS supplementations to improve chondrogenic differentiation and matrix production, providing ramifications on an adjuvant frontline approach to therapeutic intervention in cartilage tissue engineering. [Figure 4,5]

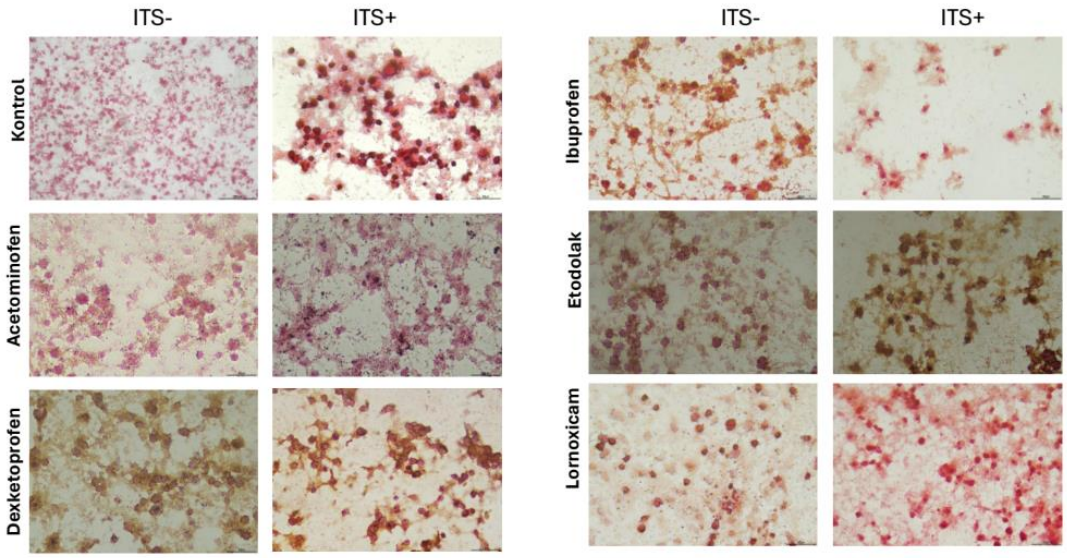


**Figure 4: INSULIN-TRANSFERRIN-SELENIUM (ITS) SUPPLEMENT OF DAY 5**

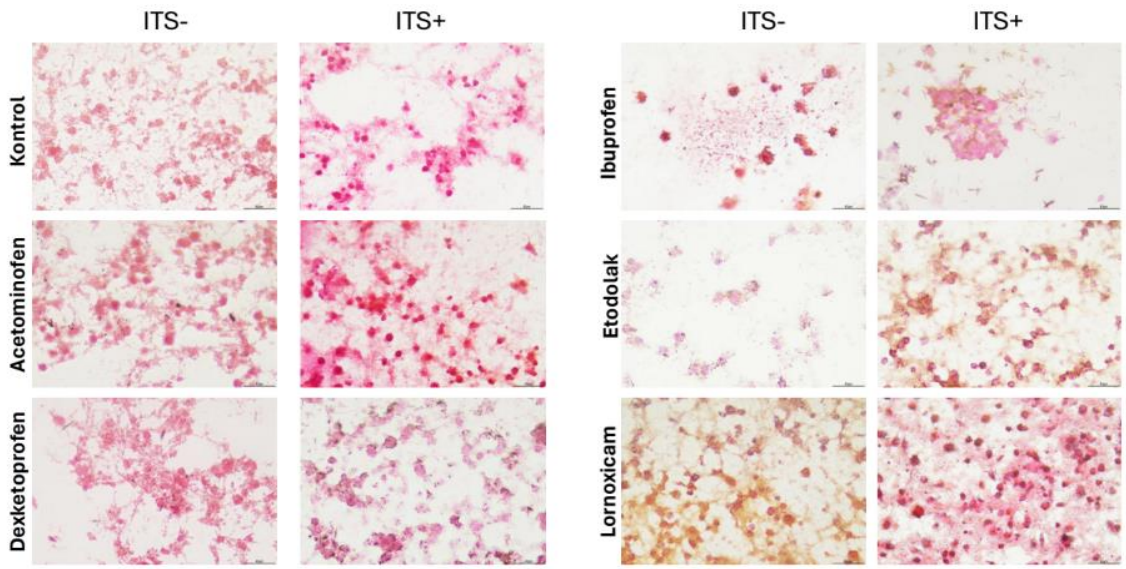


**Figure 5: INSULIN-TRANSFERRIN-SELENIUM (ITS) SUPPLEMENT OF DAY 10**

## DISCUSSİON

The present study systematically assessed the effects of several non-steroidal anti-inflammatory drugs (NSAIDs) in viability and differentiation into chondrocytes of ATDC5 cells, focusing particularly on five NSAID reference drugs: Etodolac, Dexketoprofen trometamol salt," Classic" Acetaminjsonic acid sodium salt dihydrate/Lornoxicantromethamine
hadrate/Ibuprofengel semi-solid form. The studies were designed to evaluate the effect of these drugs on chondrocyte metabolism and differentiation in a well controlled experimental model [2].

In the first series of assays, MTT test revealed that at clinically attainable concentrations, Lornoxicam, Dexketoprofen, and Ibuprofen neither significantly reduced viable cells 72 hours post-treatment. This lack of an adverse effect on mature chondrocyte cell survival may give a good sense of security with regard to the survivability of the mature chondrocytes and implied that no cytotoxic effect is induced under the current tested conditions on mature chondrocyte cells, therefore possibly relatively innocuous for the therapeutic management of diseases that required their administration [3].

We further investigated the effects of these NSAIDs on type II collagen and Aggrecan, which are critical chondrogenic markers. Specifically, they concluded that the expression levels of these genes were enhanced by Dexketoprofen, and more importantly Lornoxicam. This augmentation highlights powerful stimulatory effect on the biosynthesis of important cartilage matrix components indispensable for preserving structural as well as physiological condition in order to function properly related carilages. In contrast, although Ibuprofen and Etodolac resulted in moderate upregulation pertaining to Aggrecan expression, Acetaminophen had negligible impact when considering these gene expressions thus underscoring the limitations for this specific therapeutic intervention upon chondrogenic differentiation pathways [4,6,8].

Follow-up with Safranin-O staining, which detects glycosaminoglycan accumulation as a read-out for chondrogenetic factors, verified these results. The intensivly stained nanofibres were very prominent in case of lornoxicam, followed by dexketoprofen which further supports their better effectiveness in aiding on chondroinduction. A gradient in staining intensity among tested NSAIDs, that supports the gene expression data and furthermore visualizes different capacity of these drugs to support matrix formation in cartilage [5].

Furthermore, the study demonstrated that ITS could have an impact on chondrogenic differentiation (Fig. Aggrecan and COL2 levels were notably increased in comparison with the other drug treatment groups by supplement of ITS, which contains insulin (providing essential growth factors) transferrin (iron-binding protein required for cellular proliferation), selenium; a micronutrient that has numerous functions including antioxidation. The synergistic interaction with Lornoxicam resulted in the enhancement of chondrogenic activity to a maximal effect. This result is significant, as it provides the basis for use of ITS supplementation to boost therapeutic interventions aimed at improving cartilage repair and regeneration [7].

In summary, the results here reported evidence the diverse impact of NSAIDs on chondrogenic differentiation and supports Lornoxicam and Dexketoprofen as having a more favourable outcome. This feedback might be valuable for the demonstrating preferences to decide on NSAIDs in clinical situations where conservation or problem of cartilage honesty is needed. Furthermore, ITS supplementation increased the differentiation of CD26+ apoptotic chondro-progenitors towards a stable chondrogenic phenotype making it an attractive choice for improving therapeutic approaches on cartilage diseases and influencing the new

paradigms in cartilage tissue engineering and regenerative medicine [1].

## CONCLUSİON

In conclusion, Lornoxicam demonstrated the highest chondrogenic gene expression compared to the other evaluated NSAIDs and appears to have positive effects on chondrogenic differentiation.

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