ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Assessment of the impact of Curcumin on cell cultures derived from the primary intervertebral disc tissue in humans

İnsanlarda primer intervertebral disk dokusundan elde edilen hücre kültürleri üzerinde Curcumin'in etkisinin değerlendirilmesi

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Submitted Date: 11 August 2023, **Accepted Date:** 15 August 2023

SUMMARY

Aim: Degenerative disc disease in the lumbar spine is widely observed. Degenerative disc diseases are among the causes of low back pain in older age. Modern drug discovery studies have aimed to identify potential molecules that target multiple pathways with a safer profile against degeneration. This study aimed to evaluate the effects of curcumin, a natural phenolic compound, on primary cell cultures prepared using intervertebral disc (IVD) tissues resected during the surgeries of patients with lumbar disc herniation.

Material and Methods: Primary cell cultures were prepared using human IVD tissues of eight patients. Untreated groups served as the control and curcumin-treated groups as the study sample. In-vitro cytotoxicity analyses were performed in all groups. Acridine orange (AO)/propidium iodide (PI) and Janus Green B staining were performed to evaluate cell surface morphologies. One-way analysis of variance and Tukey HSD, a multiple comparison test, were used to assess the obtained data.

Results: Proliferation slightly increased as of 24 h in the curcumin-treated samples, but decreased in the 48 and 72 hour curcumin-treated samples compared to the control samples. The obtained results were statistically significant ($p<0.05$). Additionally no cytotoxicity was observed according to morphological evaluations.

Conclusion: This research is an in-vitro experimental study. However, this natural and non-toxic pleiotropic agent can be targeted to cell-damaged sites through appropriate drug delivery systems. It may thus be a safe treatment option for the regeneration of degenerated lumbar IVD cells in the future.

Keywords: Annulus fibrosus, curcumin, cytotoxicity, nucleus pulposus, primary cell culture, proliferation

ÖZET

Amaç: Lomber omurga bölgesinde dejeneratif disk hastalığı yaygın olarak gözlemlenmektedir. Dejeneratif disk hastalıkları, yaşlılıkta bel ağrısının nedenlerinden biridir. Modern ilaç keşfi çalışmaları, daha güvenli bir profille dejenerasyona karşı çoklu yolları hedefleyebilen potansiyel molekülleri tanımlamayı amaçlamıştır. Bu çalışmanın amacı, doğal bir fenolik bileşik olan kurkuminin, lomber disk herniasyonu olan hastaların cerrahileri sırasında çıkarılan intervertebral disk (IVD) dokuları kullanılarak hazırlanan primer hücre kültürleri üzerindeki etkilerini değerlendirmektir.

Materyal ve Metotlar: Sekiz hastanın insan IVD dokuları kullanılarak primer hücre kültürleri hazırlandı. Tedavi edilmemiş gruplar kontrol grubu olarak hizmet etti, kurkumin tedavisi uygulanan gruplar ise çalışma örneği olarak kullanıldı. Tüm gruplarda in-vitro sitotoksisite analizleri yapıldı. Hücre yüzey morfolojilerini değerlendirmek için Akrin Orange (AO)/ Propidyum İodür (PI) ve Janus Green B boyaması yapıldı. Elde edilen verileri değerlendirmek için tek yönlü varyans analizi ve çoklu karşılaştırma testi olarak Tukey HSD kullanıldı.

Bulgular: Kurkumin tedavisi uygulanan örneklerde 24 saat sonra hafif bir şekilde çoğalma görülürken, 48 ve 72 saatlik kurkumin tedavisi uygulanan örneklerde kontrol örneklerine kıyasla azaldı. Elde edilen sonuçlar istatistiksel olarak anlamlıydı ($p<0.05$). Ayrıca morfolojik değerlendirmelere göre sitotoksisite gözlenmedi.

Sonuç: Bu araştırma in-vitro deneysel bir çalışmadır. Ancak, bu doğal ve toksik olmayan çok yönlü ajan uygun ilaç taşıma sistemleri aracılığıyla hücre hasarlı bölgelere yönlendirilebilir. Bu nedenle gelecekte dejeneratif lomber IVD hücrelerinin rejenerasyonu için güvenli bir tedavi seçeneği olabilir.

Anahtar kelimeler: Annulus fibrosis, kurkumin, sitotoksisite, nukleus pulposus, primer hücre kültürü, proliferasyon

Albayrak M, Yilmaz I, Yuzbasi MF, Akalan H, Sirin DY, Karaarslan N, Ozbek H, Ates O.
Assessment of the impact of Curcumin on cell cultures derived from the primary intervertebral disc tissue in humans.
Maltepe Tıp Dergisi 2023; 15(2): 49-55. doi: 10.35514/mtd.2023.91

INTRODUCTION

Curcumin is one of the major bioactive polyphenolic compounds obtained from the rhizomes of Curcuma longa (family Zingiberaceae), a perennial herbaceous herb with yellow flowers and pointed leaves (1). This natural phenolic compound, which has been investigated for the treatment of many autoimmune and inflammatory diseases such as rheumatoid arthritis (2), influences cell cycles such as cyclin D1 and cyclin E (3), cell apoptosis through activation of caspases and reduction of receptors in antiapoptotic genes (4).

Curcumin plays a role in the molecular mechanisms of aging and senescence of cells, and its pharmacological role, therapeutic capacity, and limitations have been examined by many studies. Curcumin is an anti-aging compound and is effective in the treatment of age-related diseases (5). In addition, it plays an important role in cell survival and cell proliferation via phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (AKT) and mammalian target of rapamycin (mTOR) pathways (6).

Curcumin inhibits gene expression levels of many inflammatory factors through oxidative stress, and it alleviates neuropathic pain and neuroinflammation (7).

Curcumin and its analogs show cytostatic activity through the oxygenated aromatic rings (8). Curcumin is a natural component with anti-inflammatory, chemopreventive, antimutagenic, antitumor, antioxidant, anticytotoxic, and neuroprotective properties, and its effects have been investigated in the treatment of many different diseases (9). Curcumin has preventive and therapeutic effects on the degeneration of cartilage-like tissues. A study has evaluated the protective effects of curcumin against sodium nitroprusside-induced chondrocyte apoptosis in rabbits, and it has been suggested that curcumin both increases the vitality of chondrocytes and has a protective effect against the damage caused by sodium nitroprusside in chondrocytes.

In addition, curcumin administration protects the extracellular matrix (ECM) synthesis and prevents the degradation of the ECM. Curcumin has a protective effect on chondrocytes and is a pharmacological potential agent that can be used both in the prevention and treatment of the pathogenesis of osteoarthritis developed due to cartilage cell damage (10).

In a study using osteoarthritic synovial cells, the combination including curcumin has shown beneficial effects against osteoarthritis characterized by pain and inflammation (11). In another study on chondrocytes derived from mouse knee cartilage, chondrocyte proliferation, viability, and cytotoxicity analysis of curcumin have been performed using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay. This study has suggested that curcumin significantly increases cell viability and preserves ECM synthesis (12).

Curcumin prevents disc degeneration against oxidative damage and mitochondrial dysfunction by restoring autophagic flux in Intervertebral disc (IVD) cells (13). Curcumin affects many important pathways, especially its protective effect, and its effectiveness in lumbar IVD degeneration (IVDD) treatments has been investigated (14).

The present study aimed to in-vitro evaluate the effects of curcumin applied to primary cell cultures prepared using the degenerated IVD tissues obtained from patients with lumbar disc herniation on the viability and proliferation of annulus fibrosus (AF)/nucleus pulposus (NP) cells and ECM structures.

MATERIALS AND METHODS

Ethics approval and consent to participate

Approval has been obtained from the local ethics committee of the School of Medicine of XXX University (date: 23/02/2022 no. 43) to conduct the study. All patients signed an informed consent form.

Case selection criteria

Patients who presented to the neurosurgery clinic with complaints of low back and leg pain, loss of sensation, and urinary incontinence were evaluated. Following neurological, electrodiagnostic, and radiological examinations, patients with spinal cord and radicular compression due to lumbar disc herniation were operated on. Primary cell cultures were prepared using the tissues obtained from the patients who underwent lumbar microdiscectomy (15). In the lumbar spinal MRI examinations of the cases, discopathy level, narrowing of the disc space, annular tear and loss of density were observed, and lumbar hernia NP was present in the degenerative background. To assess the degree of IVD degeneration, the Pfirrmann classification grading system with the aid of a T2-weighted magnetic resonance image was used. IVD tissues were obtained from patients (Pfirrmann grading scale stage III-IV; 4 females, 4 males; mean age: 42.98 ± 2.18 years) without smoking history and alcohol consumption. In addition to pregnancy, those with a diagnosis of malignancy and those using biological agents were not used for the preparation of primary cultures in this study and were not included in the study.

Preparation of primary cell cultures

Cell culture protocol was performed according to the descriptions from previous studies (16, 17). Tissues were first treated mechanically and then enzymatically. For this; subsequently, 0.375 mg collagenase type II enzyme (Gibco; Thermo Fisher Scientific, Inc.) dissolved in DMEM were added and incubated with 5% CO2 at 37˚C overnight (16, 17). The samples were subsequently centrifuged at 4˚C and 161 x g for 10 min (16, 17). Cell pellets were resuspended by adding the cell culture medium, transferred to T75 flasks, and incubated at 37˚C for 72 h to obtain the primary cell cultures (16, 17). Following incubation, cells were trypsinized with trypsin EDTA (0.25%). The samples were centrifuged twice consecutively, and the supernatant on the tubes was discarded. Pellets precipitated at the bottom of the tubes were resuspended with a freshly prepared cell culture medium. The samples were transported to flasks. The cells were trypsinized and counted using the Thoma slide. The counted cells were plated at 1.5x106 cells per well in 96-well plates for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability and proliferation and toxicity assays. The cells were plated at 2.1x105 per well in 24-well plates for acridine orange (AO) and propidium iodide (PI) (AO/PI) assays, and Janus Green B assays.

Application of curcumin to human primary chondrocyte cultures

Cell samples that were not administered drugs constituted the control group samples, while the curcumin-treated group constituted the experimental group. The concentration of curcumin treated to cell cultures was 10 µM. Curcumin dissolved in 0.1% dimethyl sulfoxide (DMSO) in PBS. The Control group was treated with 0.1% DMSO in Phosphate-buffered saline (PBS) (18).

Morphological evaluation via inverted light microscopy

Cell surface morphologies and ECM structures were examined under 4×, 10×, 20×, and 40× magnifications using an inverted light microscope. Before and after the curcumin administration, the primary cell cultures were evaluated using an inverted light microscope (Olympus CKX41). To determine cell viability and confirm MTT results, nucleic acid-binding dyes AO and PI were used (19, 20). The samples were treated with a 10 µM concentration of curcumin. To prepare the AO/PI stain, AO (4 mg dissolved in 2 mL 99% ethanol), sodium-ethylenediaminetetraacetic acid (10g), PI (4 mg), and 50 mL fetal bovine serum were mixed well, and sterile distilled water was added to reach a 200 mL final volume, according to a previously reported method (20).

Leica DM 2500 fluorescent microscope was used for AO/PI analysis. The images were evaluated using the Cytovision Capture Station imaging software (version 7.0; Genetix; Leica Microsystems, Inc., Buffalo Grove, IL, USA). In addition, Janus Green B dye, which changes color according to the amount of oxygen was used to stain the mitochondria supravitally. The indicator oxidizes to a blue color when oxygen is present. In the absence of oxygen, the indicator decreases and the color changes to pink (21, 22).

Cell viability, toxicity, and proliferation analyses using MTT and enzyme-linked immunosorbent assay

The viability tests were performed using a commercial kit [3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT), Vybrant MTT Cell Proliferation Assay, Cat#V13154, Thermo Fisher Scientific, Waltham, MA, USA.)]. For viability and cell toxicity measurements, a Mindray MR 96 ELISA device (China), was used. Absorbances were measured at a wavelength of 540 nm. The viability of the control group before contrast agent addition was accepted as 100%. The proliferation and the inhibition of the proliferation were calculated using the following formulas, according to a previously reported method (20). Since the half-life of curcumin is between 5-7 h (23), all analyses were terminated within 72 h.

Statistical analysis

Minitab software (version 22) was used for the statistical evaluation. Data were evaluated at a 95% confidence interval (CI). Analysis of variance (ANOVA) was used to determine the differences between groups. Tukey's honest significant difference (HSD) test was used to assess differences between multiple groups. The results were presented as mean ± standard deviation (mean ± SD). The $p < 0.05$ was accepted as statistically significant.

RESULTS

Curcumin-treated AF and NPC cultures were followed up for 72 h. Curcumin administrated AF and NP cells preserved their morphological structures. The samples stained with Janus-Green-B dye in all experimental groups were evaluated. AO and PI staining were carried out to determine whether there was cell death in all cultures and to confirm MTT cell viability, toxicity, and proliferation analysis. Photographs of inverted microscopy, Janus Green and AO/PI-stained cultures are given in Figure 1. As can be clearly observed in Figure, AF/NPC cultures preserved their unique morphology. furthermore, AO/PI staining confirmed that there was no toxic effect or cell death at the applied dose of curcumine.

Maltepe tıp derg. Cilt: 15 Sayı: 2/2023
https://doi.org/10.35514/mtd.2023.91

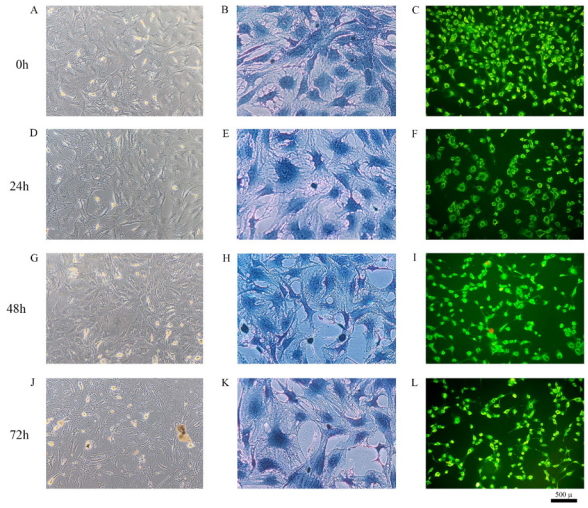

Figure 1. Morphological examinations. First column, inverted microscopy (A, D, G, and J respectively, 10× magnification); second column, Janus Green-stained cultures (B, E, H, and K respectively, 20× magnification); third column, acridine orange/propidium iodide-stained (C, F, I and L respectively,10× magnification) cultures.

Although no morphological change occurred, MTT analysis revealed a different situation. Figure 2 shows the graph of absorbance values obtained by MTT analysis (Figure 2).

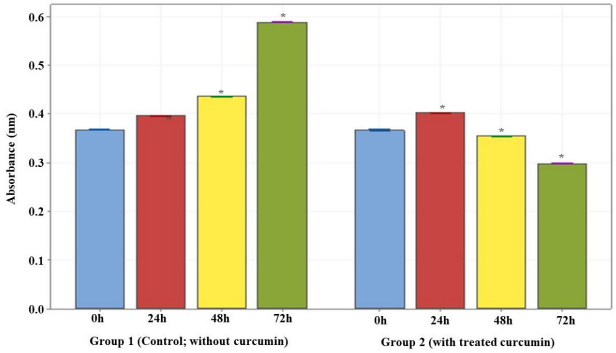

Figure 2. Interval plot of absorbance (540 nm, O.D.) comparison of the experimental group treated with curcumin compared to the control group.

As a result of the % vitality calculations; proliferation increased by 1.51% at 24 h in the curcumin-treated samples compared to the untreated samples. In turn, proliferation decreased by 18.80% and 49.31% at 48 and 72 h, respectively, in the curcumin-treated samples compared to the control samples. Statistical analysis of MTT data is given in tables (Table 1, Table 2). These results were statistically significant ($p<0.05$).

Table 1. Statistical analysis of MTT-ELISA

Source	Adj SS	Adj MS	F-Value	*p**
Groups	0.100650	0.100650	102356.02	0.000
Time (hours)	0.035656	0.011885	12086.86	0.000
Groups Time (hours)	0.171930	0.057310	58281.44	0.000

**p<0.05 vs. control group and p<0.05 vs. curcumin-treated group. Adj SS, adjusted sum of squares; Adj MS, adjusted mean square*

Table 2. Data were analyzed using a one-way analysis of variance followed by a post-hoc Turkey Pairwise Comparison test

Variable	Time (hours)	Mean±StDev	Grouping
Control	0	0.367±0.001	E
	24	0.396±0.0009	D
	48	0.436±0.0009	B
	72	0.588±0.0009	A
Curcumin treated	0	0.367±0.001	E
	24	0.402±0.0009	C
	48	0.354±0.0009	F
	72	0.298±0.0009	G

**A: Highest rate of cell viability and proliferation. G: The lowest rate of cell viability and proliferation. StdDev: standard deviation*

DISCUSSION

Inflammatory response and neoangiogenesis are induced by triggering ECM degradation, resulting in NP inhibition and accelerated development of IVDD. Lumbar IVDD is an independent risk factor for back pain, disc hernia, myelopathy, and radiculopathy (24).

Intervertebral disc disease is a complex chronic disease of the spine, and the lumbar region is significantly affected. Cervical spondylosis are the pathologies with the highest morbidity among IVD-related pathologies (14). In addition, up to 80% of adults suffer from low back pain-related diseases and lumbar IVDD has become an important health problem (14, 24). Many studies have investigated different natural compounds for the regeneration of degenerated AF/NP cells that cause such health problems. Curcumin is a non-toxic and anti-inflammatory compound and alleviates lumbar radiculopathy by reducing neuroinflammation, oxidative stress, and nociceptive factors. Therefore, this natural phenolic compound has gained popularity.

Xiao et al (18) reported that curcumin, covering a three-month treatment period, did not have acute cytotoxicity at doses of 2 g/kg body weight in mice and 8000 mg/day in humans. A study by Kakiuchi et al. tested curcumin and the authors reported that pharmacological inhibition of mTORC1 may protect against apoptosis, aging, and ECM catabolism at the cellular level in humans intervertebral disc tissue through Akt and autophagy induction. They also suggested that curcumin is not cytotoxic compared to other MTORC1 inhibitors, but its protective effect is weak (25). Cherif et al (26) aimed to reduce neuroinflammation, oxidative stress, and nociceptive factors with pharmacological agents that exhibit senolytic and anti-inflammatory activities such as curcumin.

Many studies have investigated pharmacological treatment methods not only to prevent IVDD but also to provide IVD regeneration. Cell cultures obtained from humans (14, 27) and animal tissues (13) are generally used in studies investigating the effects of curcumin on lumbar IVDD. The effects of curcumin on regeneration are investigated using commercial cell lines (28).

However, it is very well known that commercial cell lines contain only a single cell type and do not have complex coordination mechanisms in the cells' microenvironment (29). Commercial cell lines do not have the same genotypic and phenotypic characteristics as seen in the human body. Therefore, the results obtained from studies using cell lines may be misleading. The sensitivity of animal tissue is known to differ from that of human tissue (29). Results of studies using animal tissues may differ from those using human tissues, giving rise to misleading results (30). In the present study, primary cell cultures prepared from human degenerated IVDs were used. This may enhance the value of the study.

Intervertebral Disc Disease is characterized by cellular, structural, compositional, and mechanical changes in AF/NP, and is usually associated with back and low back pain. It affects not only AF/NP but also the cartilaginous end-plate adjacent to AF/NP. The endplate, which has a cartilaginous structure, changes with aging and degeneration. Tissue hydration is adversely affected due to increased calcification, decreased glycosaminoglycan/collagen, and proteoglycan levels. Therefore, permeability and diffusion-related transport of the solute may be limited (31). Some clinical patient monitoring studies have suggested that physical medicine and rehabilitation, one of the current conservative treatment modalities, has anabolic effects characterized by cell growth, proliferation in articular cartilage tissue, and an increase in ECM structure (32). However, no studies have used human IVD tissues when evaluating the effects of treatment modalities on IVDD (31). IVDD is a multifactorial, progressive process characterized by phenotypic and genotypic changes leading to low back pain and functional loss. The prolonged imbalance between anabolism and catabolism in the discs alters the microstructure at the cellular level, causing progressive proteoglycan loss and dehydration leading to IVDD.

Current treatment algorithms for IVDD may only alleviate symptoms but do not target the underlying degenerative process and its management. Many studies have investigated the differences between aging and degeneration of disc tissue and aimed to identify various factors responsible for disc degeneration and determine regeneration strategies. None of the current treatment modalities have successfully addressed the underlying biological problem symptomatic degenerated disc (33).

Organic compounds with a molecular weight of fewer than 900 Daltons are at the forefront of new trend research, with the belief that they can prevent the degeneration of intervertebral disc tissue cells and increase the regeneration of IVD due to their anti-inflammatory, anti-apoptotic, anti-oxidative and anabolic effects. Using a rat tail puncture-induced model of IVDD, a study has established that quercetin, a plant flavonol from the flavonoid group of polyphenols, has a protective effect against IVDD. In addition, quercetin can prevent IVDD by modulating autophagy due to its positive effects on cell proliferation, and, therefore, is a potential therapeutic strategy for IVDD treatment (34). Likewise, berberine, a quaternary ammonium salt of the protoberberine group of benzylisoquinoline alkaloids, suppresses the microtubule-associated protein 1A/1B-light chain 3 (LC3) protein, which plays an important role in autophagy, thus preventing apoptosis and ECM degradation in NP cells and healing cell proliferation and IVDD (35).

Cyanidin, a natural anthocyanidin type organic compound found in many plants, including grapes, blueberries, blackberries, and cherries, could alleviate apoptosis of NP cells and IVDD in vitro and in vivo studies in rats (36). In

addition, apigenin, found in many plants, and a natural product belonging to the flavone class, is known to be used in the treatment of IVDD due to its positive effects on the viability of NP cells (37).

In the present study, curcumin, an herbal and small phenolic molecule, was applied to IVD cell cultures. Proliferation increased at 24 h in the curcumin-treated samples compared to the control samples. However, it gradually decreased at 48 and 72 h ($p<0.05$). Since the curcumin compound has a very short half-life of 5-7 h, it was applied to cell cultures once and no further curcumin application was performed within 72 h. This may cause a decrease in the proliferation level. In vitro and in vivo studies generally have some limitations (38). The limitation of our study is that it was performed in primary cell cultures. Therefore, the data obtained will not fully reflect the possible systemic effects of curcumin. Curcumin, a hydrophobic polyphenol, has been shown to cause poor systemic bioavailability from the time of oral administration because of rapid metabolism and conjugation in animal studies. In addition, curcumin is absorbed after oral dosing in humans and can be detected as glucuronide and sulfate conjugates in plasma (39). Also, primary cultures were prepared from tissues obtained from the same race and a small number of people.Cell culture samples prepared from intact tissues could not be included in the study as an additional control group, since healthy disc tissue cannot be obtained within ethical frameworks.

CONCLUSION

Curcumine did not cause a decrease in cell proliferation from the time of administration until 24 h but reduces cell proliferation at 48 and 72 hours. therefore, considering the short half-life of the drug, in clinical applications appropriate drug delivery systems that will provide drug release every 24 hours may have positive effects on the viability and proliferation of AF and NP cells.

Author Contributions: Working Concept/Design: IY, NK, ÖA, Data Collection: MFY, HA, HO, Data Analysis / Interpretation: DYS, Text Draft: IY, MFY, HA, NK, HO, Critical Review of Content: MA, IY, MFY, DYS, NK, OA Final Approval and Responsibility: IY, HA, DYS, HO, Material and technical support: IY, MFY, Supervision: DYS, HO, OA
Conflict of Interest: The authors state that there is no conflict of interest regarding this manuscript.
Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Degot P, Huber V, Touraud D, Kunz W. Curcumin extracts from Curcuma Longa - Improvement of concentration, purity, and stability in food-approved and water-soluble surfactant-free microemulsions. Food Chem.2021; 339:128140.
2. Mohammadian Haftcheshmeh S, Khosrojerdi A, Aliabadi A, Lotfi S, Mohammadi A, Momtazi-Borojeni AA. Immunomodulatory Effects of Curcumin in Rheumatoid Arthritis: Evidence from Molecular Mechanisms to Clinical Outcomes. Rev Physiol Biochem Pharmacol.2021;179:1-29.
3. Meena R, Kumar S, Kumar R, Gaharwar US, Rajamani P. PLGA-CTAB curcumin nanoparticles: Fabrication, characterization and molecular basis of anticancer activity in triple negative breast cancer cell lines (MDA-MB-231 cells). Biomed Pharmacother.2017;94:944-54.
4. Zhu G, Shen Q, Jiang H, Ji O, Zhu L, Zhang L. Curcumin inhibited the growth and invasion of human monocytic leukaemia SHI-1 cells in vivo by altering MAPK and MMP signalling. Pharm Biol.2020;58:25-34.
5. Zia A, Farkhondeh T, Pourbagher-Shahri AM, Samarghandian S. The role of curcumin in aging and senescence: Molecular mechanisms. Biomed Pharmacother.2021;134: 111119.
6. Khan K, Quispe C, Javed Z, et al. Resveratrol, curcumin, paclitaxel and miRNAs mediated regulation of PI3K/Akt/mTOR pathway: go four better to treat bladder cancer. Cancer Cell Int.2020; 20:560.
7. Daugherty DJ, Marquez A, Calcutt NA, Schubert D. A novel curcumin derivative for the treatment of diabetic neuropathy. Neuropharmacology. 2018;129:26-35.
8. Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer.2005;41:1955-68.
9. He HJ, Xiong X, Zhou S, et al. Neuroprotective effects of curcumin via autophagy induction in 6-hydroxydopamine Parkinson's models. Neurochem Int.2022;155:105297.
10. Zhao P, Cheng J, Geng J, et al. Curcumin protects rabbit articular chondrocytes against sodium nitroprusside-induced apoptosis in vitro. Eur J Pharmacol.2018;828:146-53.
11. Brochard S, Pontin J, Bernay B, Boumediene K, Conrozier T, Baugé C. The benefit of combining curcumin, bromelain and harpagophytum to reduce inflammation in osteoarthritic synovial cells. BMC Complement Med Ther.2021;21:261.
12. Wang P, Ye Y, Yuan W, Tan Y, Zhang S, Meng Q. Curcumin exerts a protective effect on murine knee chondrocytes treated with IL-1β through blocking the NF-κB/HIF-2α signaling pathway. Ann Transl Med.2021;9:940.
13. Kang L, Xiang Q, Zhan S, et al. Restoration of Autophagic Flux Rescues Oxidative Damage and Mitochondrial Dysfunction to Protect against Intervertebral Disc Degeneration. Oxid Med Cell Longev.2019;2019:7810320.
14. Hu Y, Tang JS, Hou SX, et al. Neuroprotective effects of curcumin alleviate lumbar intervertebral disc degeneration through regulating the expression of iNOS,

COX-2, TGF-β1/2, MMP-9 and BDNF in a rat model. Mol Med Rep.2017;16:6864-9.
15. Somay H, Karaarslan N. Sequestrectomy or microdiscectomy in patients with lumbar disc herniation. Ann Med Res.2021;26,753-8.
16. Karaarslan N, Yilmaz I, Sirin DY, Ozbek H, Kaplan, Kaya Y E, et al. Pregabalin treatment for neuropathic pain may damage intervertebral disc tissue. Exp Ther Med.2018;16:1259-65.
17. Kaya YE, Akalan H, Yilmaz I, Karaarslan N, Yasar Sirin D, Ozbek H. Evaluation of the expression and proliferation of degenerative markers in primary cell cultures obtained from human intervertebral disc tissue. Ann Med Res.2020;27:711-6.
18. Xiao L, Ding M, Fernandez A, Zhao P, Jin L, Li X. Curcumin alleviates lumbar radiculopathy by reducing neuroinflammation, oxidative stress and nociceptive factors. Eur Cell Mater.2017;33:279-93.
19. Akgun FS, Sirin DY, Yilmaz I, et al. Investigation of the effect of dipyrone on cells isolated from intervertebral disc tissue. Exp Ther Med.2019;18:216-24.
20. Karaarslan N, Yilmaz I, Ozbek H, et al. Are radio-contrast agents commonly used in discography toxic to the intact intervertebral disc tissue cells? Basic Clin Pharmacol Toxicol.2019;124:181-9.
21. Ahmad F, Alamoudi W, Haque S, Salahuddin M, Alsamman K. Simple, reliable, and time-efficient colorimetric method for the assessment of mitochondrial function and toxicity. Bosn J Basic Med Sci.2018;18:367-74.
22. Yilmaz I, Karaarslan N. Examining the effects of HMG-CoA reductase inhibitors on anabolic and catabolic signaling pathway proteins associated with degenerative disc diseaseEur Rev Clin Pharmacol Sci.2022;26:2990-3000.
23. Fança-Berthon P, Tenon M, Bouter-Banon SL, et al. Pharmacokinetics of a Single Dose of Turmeric Curcuminoids Depends on Formulation: Results of a Human Crossover Study. J Nutr.2021;151:1802-16.
24. Tan JH, Li ZP, Liu LL, Liu H, Xue JB. IL-17 in intervertebral disc degeneration: Mechanistic insights and therapeutic implications. Cell Biol Int.2022;46:535-47.
25. Kakiuchi Y, Yurube T, Kakutani K, et al. Pharmacological inhibition of mTORC1 but not mTORC2 protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism through Akt and autophagy induction. Osteoarthritis Cartilage.2019;27:965-76.
26. Cherif H, Bisson DG, Jarzem P, Weber M, Ouellet JA, Haglund L. Curcumin and o-Vanillin Exhibit Evidence of Senolytic Activity in Human IVD Cells In Vitro. J Clin Med.2019;8:433.
27. Ma T, Guo CJ, Zhao X, Wu L, Sun SX, Jin QH. The effect of curcumin on NF-κB expression in rat with lumbar intervertebral disc degeneration. Eur Rev Med Pharmacol Sci.2015;19:1305-14.
28. Nowroozi N, Faraji S, Nouralishahi A, Shahrousvand M. Biological and structural properties of graphene oxide/curcumin nanocomposite incorporated chitosan as a scaffold for wound healing application. Life Sci.2021;264:118640.
29. Karaarslan N, Yilmaz I, Sirin DY. Toxicity of the acetyl-para-aminophenol group of medicines to intact intervertebral disc tissue cells. Exp Ther Med.2021;21:147.
30. Yilmaz I, Karaarslan N, Ozbek H. Practical Performance of Hippocampal Tissue Resection in Rats in Pharmacomolecular Research. Turk Neurosurg.2021;31(1):112-8.
31. Ashinsky B, Smith HE, Mauck RL, Gullbrand SE. Intervertebral disc degeneration and regeneration: a motion segment perspective. Eur Cell Mater.2021;41:370-80.
32. Hartman RA, Yurube T, Ngo K, et al. Biological responses to flexion/extension in spinal segments ex-vivo. J Orthop Res.2015;33:1255-64.
33. Kamali A, Ziadlou R, Lang G, et al. Small molecule-based treatment approaches for intervertebral disc degeneration: Current options and future directions. Theranostics.2021;11:27-47.
34. Zhang S, Liang W, Abulizi Y, et al. Quercetin Alleviates Intervertebral Disc Degeneration by Modulating p38 MAPK-Mediated Autophagy. Biomed Res Int.2021;2021:6631562.
35. Chen Y, Zheng Z, Wang J, et al. Berberine suppresses apoptosis and extracellular matrix (ECM) degradation in nucleus pulposus cells and ameliorates disc degeneration in a rodent model. Int J Biol Sci.2018;14:682-92.
36. Bai X, Jiang M, Wang J, et al. Cyanidin attenuates the apoptosis of rat nucleus pulposus cells and the degeneration of intervertebral disc via the JAK2/STAT3 signal pathway in vitro and in vivo. Pharm Biol.2022;60:427-36.
37. Xie C, Shi Y, Chen Z, et al. Apigenin Alleviates Intervertebral Disc Degeneration via Restoring Autophagy Flux in Nucleus Pulposus Cells. Front Cell Dev Biol.2022;9:787278.
38. Brown GN, Sattler RL, Guo XE. Experimental studies of bone mechanoadaptation: bridging in vitro and in vivo studies with multiscale systems. Interface Focus.2016;6:20150071.
39. Vareed SK, Kakarala M, Ruffin MT, et al. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. Cancer Epidemiol Biomarkers Prev.2008;17:1411-17.