EXPERIMENTAL / BASIC SCIENCE RESEARCH

acta orthopaedica belgica, 2026, 92, online-only

doi.org/10.52628/92.3.15655

Comparative Chondroprotective Effects of Curcumin, Resveratrol,and Eggshell Membrane in a Surgically Induced Rat Osteoarthritis Model

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ABSTRACT

Natural compounds such as curcumin (C), resveratrol (R), and eggshell membrane (EM) have demonstrated anti-inflammatory and chondroprotective properties. This study aimed to evaluate the effects of these compounds, administered either individually or in combination, on cartilage and synovial tissues in a surgically induced rat osteoarthritis (OA) model. Forty-eight male Wistar rats were randomly assigned to eight groups (n = 6 per group). OA was induced by destabilization of the medial meniscus (DMM) in the right knee. After a 4-week postoperative period, C, R, EM, their pairwise combinations (CR, REM, CEM), and the triple combination (CREM) were administered daily via oral gavage for 4 weeks. A sham-operated group served as the healthy control. Histological evaluation was performed using H&E and Safranin O/Fast Green staining. OA severity was assessed using the OARSI scoring system and semiquantitative synovial parameters. All treatment groups demonstrated varying degrees of histopathological preservation within the experimental OA model. Among single-agent treatments, resveratrol demonstrated the most consistent protective profile. The triple combination group demonstrated favorable histopathological findings, including reduced cartilage degeneration and minimal synovial inflammatory changes. However, no statistically significant differences were observed between the triple combination and key monotherapy groups. The relatively small sample size may have limited the statistical power to detecting subtle intergroup differences. Resveratrol and eggshell membrane demonstrated consistent chondroprotective effects. The combination therapy was associated with favorable histological outcomes, suggesting possible additive rather than truly synergistic effects. Further studies incorporating molecular, biochemical, and functional analyses are warranted to confirm these findings.

Keywords: Osteoarthritis, curcumin, resveratrol, eggshell membrane, chondroprotection.

INTRODUCTION

Osteoarthritis (OA) is a progressive and multifactorial joint disorder characterized by cartilage degradation, synovial inflammation, osteophytem formation, and subchondral bone remodeling. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α play a central role in disease progression by promoting extracellular matrix degradation and chondrocyte apoptosis[1]. Current pharmacological treatments for OA are largely focused on symptomatic relief and may be associated with adverse effects during prolonged use[2-4]. However, their capacity to prevent cartilage degeneration or modify disease progression remains limited. As a result,increasing attention has been directed toward biologically active compounds and regenerative therapies with potential chondroprotective and disease-modifying effects[5,6]. Recent orthopaedic literatüre highlights the growing role of orthobiologics, tissue-preserving interventions, and nutraceutical-based strategies as emerging therapeutic options. These approaches are being actively investigated for their ability to preserve cartilage integrity, alleviate symptoms, and potentially influence the underlying disease process[7-10]. Resveratrol (R) and curcumin (C) are plant-derived polyphenols with well documented anti-inflammatory and antioxidant effects. Both compounds have been shown to modulate key inflammatory signaling pathways, particularly nuclear factor kappa B (NF-κB), thereby reducing cartilage degradation and chondrocyte apoptosis[11-14]. Eggshell membrane

(EM), a natural biomaterial rich in collagen, glycosaminoglycans, and bioactive proteins, has also demonstrated anti-inflammatory and joint-supportive properties[15,16]. Although the individual effects of these compounds have been investigated, evidence regarding their combined effects remains limited. In particular, it remains unclear whether combination therapy provides additional benefits beyond those achieved with individual agents. Therefore, the aim of this study was to evaluate the effects of curcumin, resveratrol, and eggshell membrane, administered either individually or in combination in a surgically induced OA model, and to determine whether combination therapy provides additional benefits over single-agent treatment.

MATERIALS (ANIMALS) AND METHODS

Forty-eight male Wistar rats (10 weeks old) were included in this study. The animals were housed under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature and humidity, and had free access to food and water. All experimental procedures were approved by the Animal Ethics Committee of Bezmialem Vakıf University (Approval No.2021/57). Anesthesia was induced with intraperitoneal xylazine (10 mg/kg) and ketamine (35 mg/kg). Prophylactic cefazolin (50 mg/kg) was administered intramuscularly prior to surgery. The destabilization of the medial meniscus (DMM) model is a well-established and extensively validated experimental model that reproducibly induces progressive osteoarthritic changes, including cartilage degeneration and synovial inflammation[17-19]. Therefore, a post-traumatic osteoarthritis model was established using the destabilization of the medial meniscus (DMM) technique via transection of the medial meniscotibial ligament through a medial parapatellar approach. Following the procedure, the joint capsule was closed with absorbable sutures, and buprenorphine (0.05 mg/kg, subcutaneous) was administered for postoperative analgesia. Animals were monitored daily under veterinary supervision.

After a 4-week postoperative period, the rats were randomly allocated into eight groups (n = 6 per group): resveratrol (R), curcumin (C), eggshell membrane (EM), curcumin + resveratrol (CR), resveratrol + eggshell membrane (REM), curcumin + eggshell membrane (CEM), curcumin + resveratrol + eggshell membrane (CREM), and a sham-operated control group. In the sham group, only joint capsule exposure was performed without DMM induction. The study was primarily designed to compare the relative histopathological effects of different treatment regimens within a standardized experimental OA model. Curcumin (≥94% curcuminoids), resveratrol (≥99%), glycerol, sodium carboxymethyl cellulose, and Tween 80 were obtained from Sigma-Aldrich. Eggshell membrane powder (99%) was obtained from Rongsheng Biotechnology Co., Ltd. C, R, and EM were administered at doses of 50 mg/kg, 45 mg/kg, and 52 mg/kg, respectively, based on previous studies[20-22]. Each formulation was prepared according to body weight in a total volume of 1 mL per compound; therefore, animals receiving one, two, or three agents received 1 mL, 2 mL, or 3 mL of suspension, respectively. All suspensions were freshly prepared one hour prior to administration and homogenized before use. Curcumin was dissolved in a mixture of 4% glycerol and 1% Tween 80 in distilled water, whereas resveratrol and eggshell membrane were suspended in 0.5% sodium carboxymethyl cellulose. Treatments were administered once daily via oral gavage for 4 weeks[23-25].

At the end of the experimental period, the animals were sacrificed, and knee joints were harvested for histopathological evaluation. Specimens were fixed in 10% neutral buffered formalin, decalcified in formic acid, embedded in paraffin, and sectioned sagittally through the medial compartment. Cartilage and synovial tissues were evaluated using hematoxylin and eosin (H&E) staining. Safranin O/Fast Green staining was used to assess cartilage matrix integrity and proteoglycan content. All histological assessments were performed by a blinded observer who was unaware of treatment allocation throughout the evaluation process. Histological grading and staging were performed on serial sagittal sections obtained

from the medial compartment of each knee joint using standardized OARSI criteria. To minimize observational variability, all specimens were evaluated under identical conditions and according to predefined scoring parameters. Osteoarthritis severity was assessed using the Osteoarthritis Research Society International (OARSI) scoring system, which grades cartilage lesion depth (0–6) and stages the extent of surface involvement (0–4)[26]. The total OARSI score was calculated as grade Å~ stage. Synovial evaluation was performed using previously described semiquantitative criteria adapted from established experimental OA studies[26]. Synovial hyperplasia was graded from 0 to 3 based on the number of synovial lining layers. Synovial vascularity was assessed at Å~400 magnification

and scored from 0 to 3 according to the proportion of the field occupied by blood vessels[27]. Additional parameters, including hemosiderin deposition, intra-articular hemorrhage, villous formation, and cartilage erosion, were recorded as present or absent.

Statistical analyses were performed using SPSS software. Due to the nonnormal distribution of the data the Kruskal–Wallis test was used for overall comparisons, followed by pairwise Mann–Whitney U tests when appropriate. Two-sided exact P values were reported for pairwise Mann–Whitney U comparisons whenever feasible. To account for multiple pairwise comparisons, P values were adjusted using the Holm correction. Given the exploratory nature of the study, limited sample size, and nonparametric distribution of the data, emphasis was placed on comparative nonparametric analyses together with effect size estimation. Effect size was estimated using rank-biserial correlation (r) to facilitate interpretation of the magnitude of intergroup differences independent of statistical significance. Rank-biserial correlation (r) values of approximately 0.10, 0.30, and 0.50 were interpreted as representing small, moderate, and large effect sizes, respectively.

DISCUSSION

The main finding of this study is that resveratrol and eggshell membrane demonstrated consistent chondroprotective effects both alone and in combination in a surgically induced osteoarthritis model. Although varying degrees of histopathological preservation were observed among treatment groups within the experimental osteoarthritis model, the triple combination did not demonstrate clear superiority over resveratrol or eggshell membrane alone, suggesting a potentially additive rather than truly synergistic effect.

Resveratrol exhibited the most stable protective profile, with histological features remaining close to the healthy baseline. This finding is consistent with previous studies demonstrating that resveratrol modulates key inflammatory pathways, particularly nuclear factor kappa B (NF-κB), and reduces chondrocyte apoptosis[11,14,24,28]. These mechanisms may explain the consistent histopathological benefits observed in the present study.

Eggshell membrane also demonstrated notable anti-inflammatory and chondroprotective effects. Its composition, which includes collagen, glycosaminoglycans, and bioactive proteins, may contribute to preserving cartilage structure and reducing synovial inflammation[15,16,29-31]. The present findings are in agreement with previous reports suggesting a stabilizing effect of eggshell membrane on joint tissue. In contrast, curcumin-treated animals showed greater variability in histological outcomes. This variability was also observed in the combination groups containing curcumin, which did not demonstrate improved outcomes compared to their corresponding monotherapy groups. The relatively variable and less consistent effects observed in the curcumin-treated group may be attributed to its well-documented limitations in bioavailability and pharmacokinetics[32-34]. Curcumin exhibits poor aqueous solubility and limited intestinal absorption, leading to low systemic bioavailability following oral administration. Furthermore, it undergoes rapid metabolism, particularly via glucuronidation, resulting in a short half-life and reduced tissue distribution. Future studies incorporating bioavailability-enhancing formulations, such asnanoparticles, liposomal delivery systems, or adjuvants, may help to better elucidate the therapeutic potential of curcumin[34]. The triple combination group demonstrated consistent histological improvement, characterized by reduced cartilage degeneration and minimal synovial inflammation. However, the absence of statistically significant pairwise differences compared to resveratrol or eggshell membrane monotherapy suggests that the combined effect may be additive rather than truly synergistic. Although previous in vitro studies have reported potential synergistic interactions between polyphenols, such effects were not confirmed in this in vivo model[13]. From a translational perspective, these findings may be relevant for earlystage osteoarthritis, where disease-modifying treatment options remain limited. Current orthopaedic research increasingly emphasizes joint preserving and disease-modifying approaches in osteoarthritis management, including biomechanical and biologic treatment strategies[35]. Growing interest in these therapies reflects the ongoing need for interventions capable of slowing cartilage degeneration and modulating disease progression in osteoarthritis[7]. Multi-compound approaches targeting different inflammatory and degenerative pathways may offer theoretical advantages. In parallel, regenerative biologic therapies such as mesenchymal stem cell applications are increasingly being investigated for cartilage preservation and symptom modulation in osteoarthritis[10]. However, the lack of clear superiority over key monotherapies indicates that the potential clinical benefit of combination therapy should be interpreted with caution.

This study has several limitations. First, an important limitation of the present study is the absence of a dedicated untreated DMM-induced osteoarthritis control group, which may limit direct interpretation of treatment-specific efficacy. However, the DMM model used in the present study is a well-established, extensively validated, reliable and reproducible experimental model of osteoarthritis, and the primary aim of this study was to comparatively evaluate the relativehistopathological effects of different treatment regimens rather than to reconfirm OA induction itself. Future experimental studies should systematically include an untreated DMM control group to permit assessment of absolute treatment efficacy in addition to comparative efficacy between different interventions. Second, the relatively small sample size may have limited the statistical power for detecting subtle intergroup differences particularly among combination treatment regimens. Third, the experimental model represents posttraumatic osteoarthritis and may not fully reflect the complexity of human disease. Fourth, the treatment duration was relatively short, and the long-term effects remain unclear. In addition, the study relied primarily on histopathological evaluation without molecular or functional outcome measures. Moreover, the doses used in this experimental rat model cannot be directly extrapolated to clinical supplementation because of important pharmacokinetic differences between species, and dose translation to human-equivalent levels was not assessed. Fifth, no formal intraobserver reproducibility analysis was performed. Nevertheless, all histopathological evaluations were conducted by a single blinded observer using predefined OARSI criteria under standardized conditions to minimize observational variability. Future studies incorporating larger sample sizes, molecular analyses, functional assessments, and longer follow-up periods are warranted to better define the mechanisms and clinical relevance of these findings.

CONCLUSION

Resveratrol and eggshell membrane demonstrated consistentchondroprotectiveeffectsinthisexperimental osteoarthritis model. Although combination therapy was associated with favorable histopathological outcomes, these findings did not provide sufficient evidence of additive or synergistic effects beyond those achieved with individual treatments. Therefore, the present findings should primarily be interpreted as comparative histopathological observations within the limitations of the current experimental design. Further experimental and clinical studies incorporating molecular and functional outcome analyses are required to better define the translational relevance and therapeutic potential of these compounds.

Funding: This study was supported by the Bezmialem Vakıf University Scientific Research Project (Project No. 20210612).

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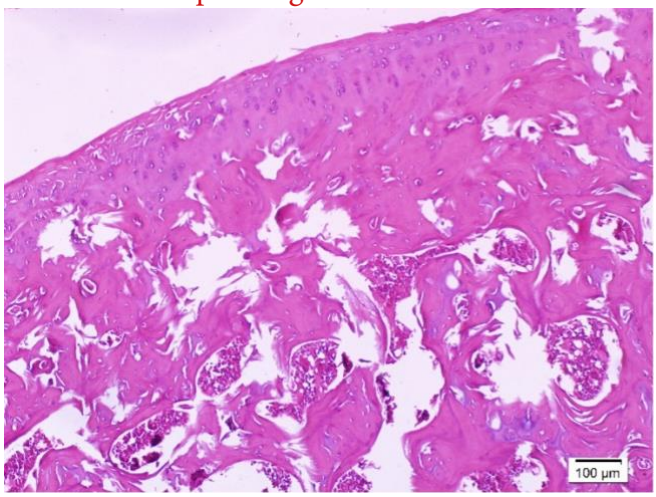

Fig. 1 — Representative histological section from the resveratrol (R) group demonstrating mild cartilage degeneration characterized by surface discontinuity and fibrillation, consistent with Grade 2 osteoarthritic changes. Minimal synovial inflammatory changes were observed. Hematoxylin and eosin (H&E) stain, ×10 magnification.

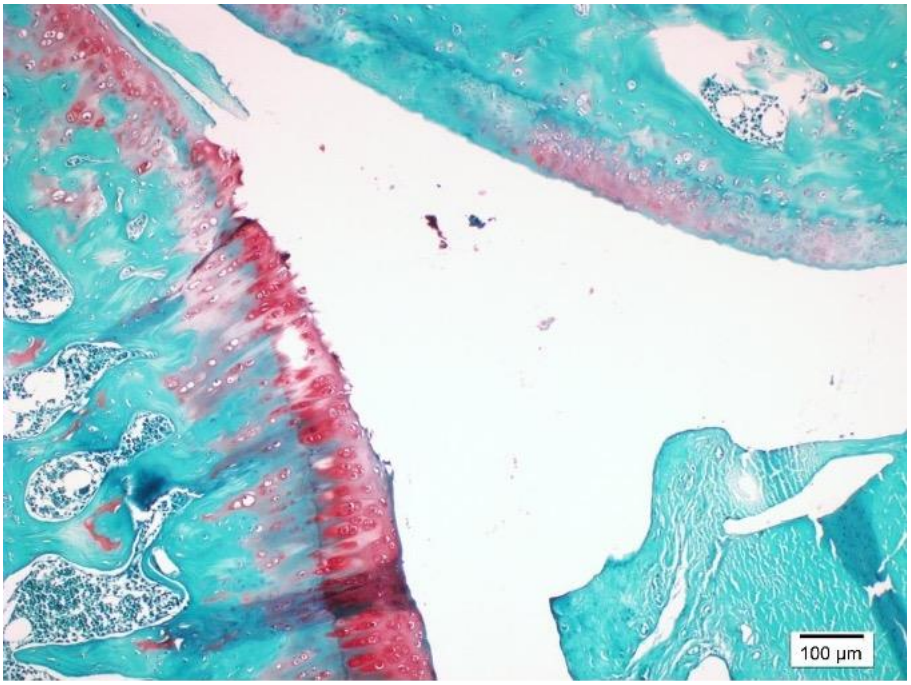

Fig. 2 — Representative Safranin O/Fast Green-stained section from the curcumin (C) group demonstrating mild-to-moderate cartilage degeneration with partial loss of proteoglycan staining and surface irregularity. Residual preservation of cartilage matrix architecture is evident. Safranin O/Fast Green stain, ×10 magnification.

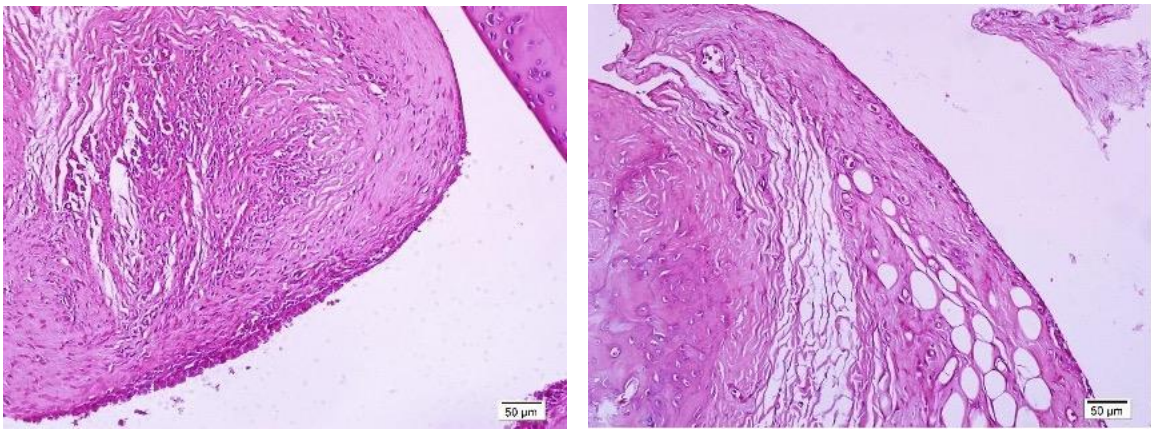

Fig. 3a & b — Representative synovial histopathological findings in experimental osteoarthritis. (a) Synovial hyperplasia characterized by increased cellularity and thickening of the synovial lining layer (arrows). (b) Increased synovial vascularity with newly formed blood vessels (arrows). Hematoxylin and eosin (H&E) stain, ×20 magnification.

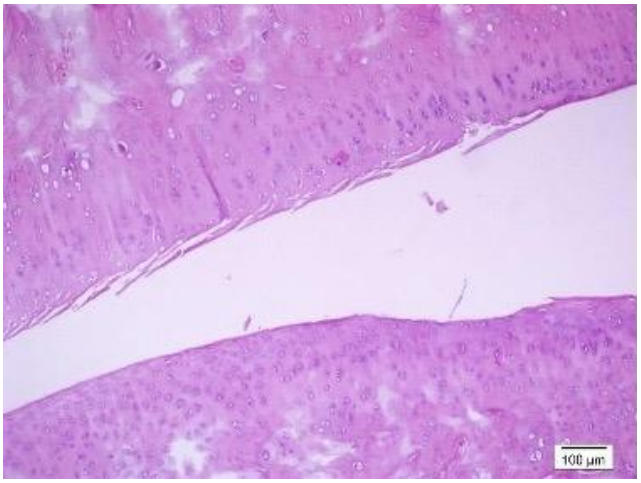

Fig. 4 — Representative histological section from the curcumin + resveratrol + eggshell membrane (CREM) group demonstrating limited cartilage degeneration with surface discontinuity and focal deep fissuring corresponding to Grade 2 osteoarthritic changes. Synovial inflammatory changes were minimal, with preservation of overall cartilage architecture. Hematoxylin and eosin (H&E) stain, ×10 magnification.

Table I. — Histopathological characteristics of the experimental groups.

Group	Description	Grade	Stage	OARSI Score Median (IQR)	Mean Synovial Findings Score Median (IQR)	Cartilage Erosion Median (IQR)	Key observations
Sham	No DMM; baseline health	Grade 1	Stage 1	1 (1–1)	0.08 (0.00–0.17)	0 (0–0)	Near-normal joint health, minimal inflammation.
R	Mild OA with consistent protection	Grade 1	Stage 1–2	1.5 (1–2)	0.50 (0.38–0.63)	0 (0–0)	Strong chondroprotective effects; low variability.
EM	Mild OA, stable	Grade 1	Stage 2–3	2.5 (2–3)	0.50 (0.50–0.63)	0 (0–0)	Excellent anti-inflammatory effects; no cartilage erosion.
C	Mild-to-moderate degeneration	Grade 1–3	Stage 2–3	3.5 (3–4)	1.17 (1.17–1.67)	0.5 (0–1)	Overall protective effects; one specimen showed severe degeneration, whereas the remaining specimens demonstrated mild changes.
REM	Moderate protection	Grade 1–2	Stage 2–3	3.5 (3–4)	0.50 (0.50–0.63)	0 (0–0)	Mild-to-moderate degenerative changes.
CR	Moderate and inconsistent protection	Grade 1–2	Stage 2–3	3.5 (2.5–5.5)	1.08 (0.88–1.17)	0.5 (0–1)	Occasional inflammatory findings with moderate histopathological preservation.
CEM	Moderate protection	Grade 1–2	Stage 2–3	3.5 (3–5.5)	1.33 (1.21–1.46)	0.5 (0–1)	Varying degrees of degeneration; no strong synergy.
CREM	Most favorable results	Grade 1	Stage 2–3	2.5 (2–3)	0.25 (0.17–0.33)	0 (0–0)	Most favorable histopathological preservation.

Qualitative OARSI grade and stage classifications are presented together with quantitative histopathological outcomes. OARSI Score, Mean Synovial Findings Score, and Cartilage Erosion are reported as median (interquartile range). Mean Synovial Findings Score was calculated as the arithmetic mean of synovial hyperplasia, vascularity, hemosiderin deposition, intra-articular hemorrhage (blood), villous formation, and synovial reaction. DMM, destabilization of the medial meniscus; OARSI, Osteoarthritis Research Society International.

Table II. — Pairwise comparisons of synovitis and cartilage scores between the sham group and each treatment group.

Group vs Sham	p (Synovitis)	r (Synovitis)	p (Cartilage)	r (Cartilage)
R	1.000	0.00	0.465	0.17
C	0.618	0.20	0.223	0.33
EM	0.762	−0.13	0.465	0.17
CR	0.762	0.13	0.221	0.33
REM	0.434	−0.32	0.180	0.40
CEM	1.000	0.00	0.223	0.33
CREM	1.000	0.03	0.223	0.33

Pairwise comparisons were performed using the Mann–Whitney U test. Effect sizes were estimated using the rank-biserial correlation (r), with values of approximately 0.10, 0.30, and 0.50 interpreted as small, moderate, and large effect sizes, respectively. Positive r values indicate higher scores in the treatment group relative to the sham group, whereas negative r values indicate lower scores in the treatment group relative to the sham group.