

Resveratrol and prednisolone loaded into human serum albumin nanoparticles for the alleviation of rheumatoid arthritis symptoms: an in vitro and in vivo study

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Rheumatoid arthritis is a chronic autoimmune-disease-causing inflammation, joint pain, and joint destruction, severely affecting the quality of life of millions worldwide. In the current research, a nanocarrier system was developed for the delivery of resveratrol and prednisolone to treat rheumatoid arthritis. The drug delivery system was characterized in vitro using scanning electron microscopy and various cell culture studies. Finally, the alleviative symptoms of the developed treatment strategy were investigated in a rat model of rheumatoid arthritis. In vitro studies showed that the carrier system released the drugs in a sustained manner and possessed strong immunomodulatory functions. Nanocarriers loaded with prednisolone, resveratrol, and drug-free carriers had 396.88 ± 76.41 nm, 392.49 ± 97.31 nm, and 338.02 ± 77.75 nm of mean particle size, respectively. In vivo studies revealed that local injection of the carrier system could alleviate the degenerative effects of rheumatoid arthritis. ELISA assays showed that the co-injection of resveratrol and prednisolone-loaded albumin nanoparticles could significantly modulate inflammatory responses. The developed treatment modality may potentially be used to treat rheumatoid arthritis.

Keywords: *resveratrol, prednisolone, rheumatoid arthritis, albumin nanoparticles*

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune-disease-causing inflammation, joint pain, and joint destruction, severely affecting the quality of life of millions worldwide. Current treatments like NSAIDs and DMARDs have limitations and adverse effects. Hence, innovative therapies are crucial [1–3].

Recent advancements in nanomedicine have opened up promising avenues for enhancing drug delivery and augmenting the therapeutic efficacy of various medications [4, 5]. Among the candidates showing promise, Resveratrol and Prednisolone, known for their potent anti-inflammatory and immunomodulatory properties, have emerged as potential agents for RA management [6, 7].

Resveratrol is a natural polyphenol found in various dietary sources, including grapes, red wine, and peanuts [6]. It is known for its antioxidant and anti-inflammatory properties. Extensive research has shown that resveratrol can inhibit the activity of inflammatory enzymes and cytokines associated with RA, thus mitigating the inflammatory processes responsible for joint damage and pain [8–10]. Furthermore, resveratrol can enhance the activity of the body's endogenous antioxidant systems, providing a multi-faceted approach to combat the oxidative stress often associated with RA [11]. Prednisolone, on the other hand, is a synthetic glucocorticoid used extensively in RA treatment for its potent immunosuppressive and anti-inflammatory effects. It functions by suppressing the immune system's inappropriate response and the release of pro-inflammatory mediators, providing rapid relief

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from RA symptoms [12–14]. However, its long-term use is associated with serious side effects such as osteoporosis, weight gain, and hypertension, underscoring the importance of developing more targeted delivery systems to limit its systemic exposure [15].

Nevertheless, the clinical utility of these drugs is constrained by challenges related to bioavailability, systemic side effects, and rapid clearance from the body [16]. Albumin nanoparticles (ALNPs) have gained prominence as an ideal drug delivery system due to their biocompatibility, non-immunogenicity, and versatility in encapsulating a wide range of therapeutic compounds [16, 17]. The aim of the current research is to load ALNPs with both prednisolone and resveratrol and assess their ability to modulate inflammatory responses for treating RA. This is the first study developing a carrier system for prednisolone and resveratrol using ALNPs to treat RA.

2. Methods and materials

2.1. Preparation of prednisolone and resveratrol-loaded ALNPs

Prednisolone-loaded ALNPs (PREALNPs) and resveratrol-loaded ALNPs (RESALNPs) were prepared using a method as described before [18]. Briefly, 500 mg of bovine serum albumin (BSA) was added to 5 ml of Milli-Q and mixed with a magnetic stirrer for 15 minutes at room temperature. Next, 20 ml of ethanol containing 2 wt.% prednisolone or 2 wt.% resveratrol was slowly added to the BSA solution and thoroughly mixed until the solution changed color. Then, glutaraldehyde solution was added to the mixture at 8 v/v% and mixed overnight. The solution was centrifuged at 15,000 rpm for 45 minutes, and the pellet was dispersed in Milli-Q water, followed by sonication (5 minutes) and lyophilization (48 hours). The nanocarrier powder was kept at 4°C until further use.

2.2. Scanning electron microscopy

PREALNPs and RESALNPs were first lyophilized and then coated with gold for 250

seconds, and then they were analyzed under 25 kV accelerating high voltage.

2.3. Dynamic light scattering (DLS) assessment

Various characteristics of PREALNPs and RESALNPs, including particle size and zeta potential utilizing dynamic light scattering (Zetasizer Nano ZS90, Malvern Instruments, UK)

2.4. Cell viability assay

The viability of MG63 cells cultured with different concentrations of PREALNPs and RESALNPs was assessed using an MTT assay kit (Abcam, USA). Briefly, 7,000 cells were cultured in 96 well-plates with DMEM (Invitrogen, USA) culture media containing 10% FBS (Invitrogen, USA), 1% antibiotics (Sigma Aldrich, USA), and different concentrations of nanoparticles (25 µg/ml, 50 µg/ml, and 100 µg/ml) for 7 days. On days 3, 5, and 7, cell viability was assessed using the instructions provided in the kit.

2.5. Anti-inflammatory assay

The impact of PREALNPs and RESALNPs on the release of key inflammatory cytokines such as IL6, IL-1 β , and TNF- α was investigated. In a nutshell, J774A1 cells obtained from the Pasteur Institute in Tehran, Iran, were grown in the presence of varying nanocarrier concentrations (25 µg/ml, 50 µg/ml, and 100 µg/ml) and cultured for a period of 48 hours. Following this, macrophage cells were activated by the addition of 1 µg/ml LPS for a duration of 12 hours. Ultimately, the concentration of cytokines was determined using an ELISA kit from Abcam as described by the manufacturer.

2.6. Release assay

The evaluation of resveratrol and prednisolone release from PREALNPs and RESALNPs involved the dissolution of 100 mg of each nanocarrier sample in 15 ml of PBS, followed by a 14-day incubation period. At various time intervals, 0.5 ml of PBS was sampled, and the absorbance was measured at 305 nm and 245 nm for resveratrol and

prednisolone, respectively. Subsequently, an equivalent volume of fresh PBS was introduced into the release medium. The obtained optical density data points were then applied to the standard curves for resveratrol and prednisolone in PBS, enabling the calculation of cumulative drug release.

2.7. In vivo study

In order to induce a rheumatoid joint model, 5 $\mu\text{g/kg}$ of Complete Freund's adjuvant (CFA) with type II bovine collagen (both purchased from Sigma Aldrich, USA) were injected into the paw joint of Male Wistar rats three times during a 10-day interval. Then, the animals were randomly divided into four groups as follows: 1 – PREALNPs in which the animals received oral gavage of PREALNPs (400 $\mu\text{g/kg}$ in normal saline solution), 2 – RESALNPs in which the animals received oral gavage of RESALNPs (400 $\mu\text{g/kg}$ in normal saline solution), 3 – Hybrid group in which the animals received oral gavage of both PREALNPs and RESALNPs (400 $\mu\text{g/kg}$ in normal saline solution), 4 – Control group in which the animals only received normal saline solution. The treatment plan continued until day 21. Then, the animals were sacrificed with ketamine overdose, and cartilage tissues were harvested for histopathological examinations using Hematoxylin and Eosin (H&E) staining and Masson's trichrome staining.

2.8. ELISA assay

After sacrificing the animals on day 21, the hind paw joint tissues were harvested, and the tissue levels of TNF- α , TGF- β , and IL-6 were assessed using an Enzyme-linked Immunosorbent Assay kit (Abcam, USA) according to the instructions provided by the manufacturer.

2.9. Statistical analysis

Data was analyzed using Graphpad prism via student's t-test and one-way ANOVA methods. All experiments were repeated at least three times.

Table 1. DLS assessment results

Sample name	Hydrodynamic size	Zeta potential
RESALNPs	$504.067 \pm 30.38 \text{ nm}$	$-26.05 \pm 6.60 \text{ mV}$
PREALNPs	$499.91 \pm 55.68 \text{ nm}$	$-24.93 \pm 9.94 \text{ mV}$
ALNPs	$536.90 \pm 42.30 \text{ nm}$	$-26.33 \pm 3.21 \text{ mV}$

3. Results

3.1. Scanning electron microscopy assay results

Results showed that PREALNPs, RESALNPs, and drug-free ALNPs had smooth surfaces with a narrow size distribution. The particles were almost round, and no signs of aggregation were observed. Particle size measurement showed that PREALNPs, RESALNPs, and drug-free ALNPs had around $396.88 \pm 76.41 \text{ nm}$, $392.49 \pm 97.31 \text{ nm}$, and $338.02 \pm 77.75 \text{ nm}$ of mean particle size, respectively.

3.2. DLS assessment results

Results (Table 1) showed that the hydrodynamic size and zeta potential of RESALNPs, PREALNPs, and drug-free ALNPs were not significantly different between groups, with p-value > 0.05 . The size measured by the DLS was bigger than the size measured by SEM imaging.

3.3. Cell viability assessment results

Results (Figure 2) showed that at 25 $\mu\text{g/ml}$ concentration, the viability of MG63 cells was not significantly different between control and RESALNPs, PREALNPs, and ALNPs groups, with p-value > 0.05 . At 50 and 100 $\mu\text{g/ml}$ concentrations; on days 5 and 7, cells cultured with RESALNPs had significantly higher cell viability than the cells cultured with PREALNPs, p-value < 0.05 .

3.4. Anti-inflammatory assay results

Results (Figure 3) showed that at all the studied concentrations, the anti-inflammatory activity of ALNPs was not significantly different from the control group, signifying the negligible immunomodulatory potential of ALNPs. At all concentrations, the immunomodulatory activity of PREALNPs was significantly higher than that of

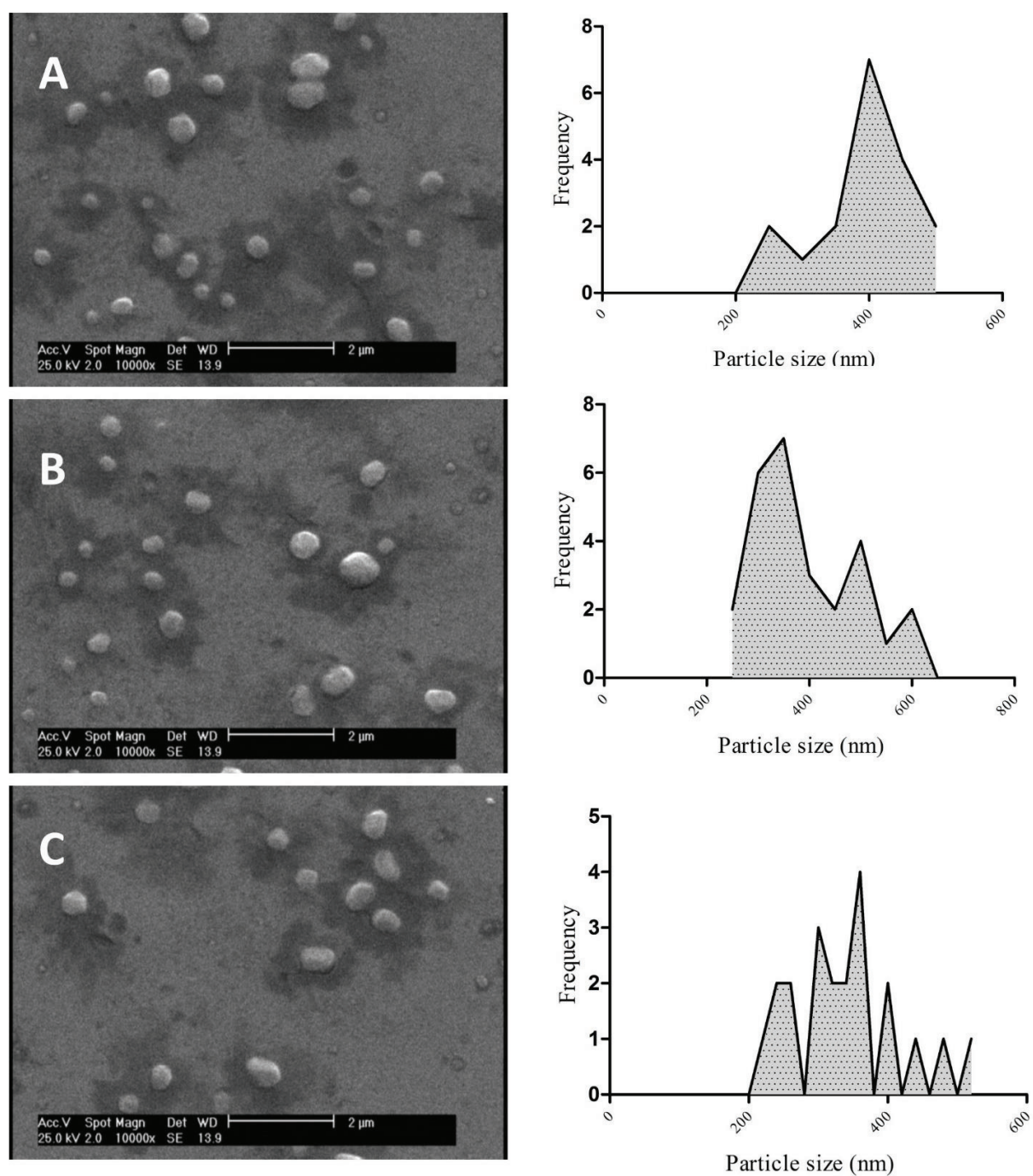


Fig. 1. SEM images of (A) RESALNPs, (B) PREALNPs, and (C) drug-free ALNPs. Right panel shows the size distribution of each nanocarrier

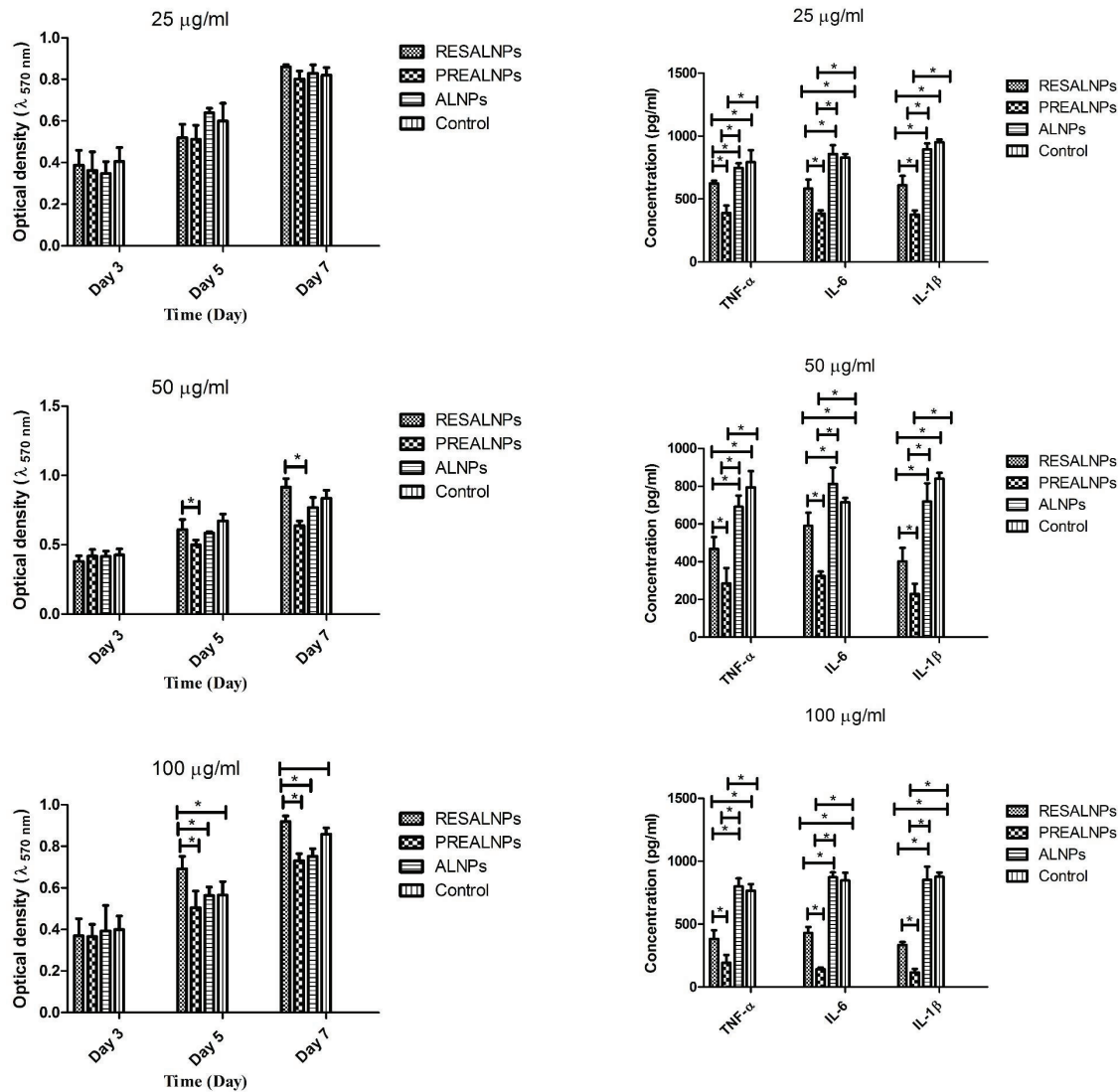


Fig. 2. MTT assay with MG-63 cells cultured with RESALNPs, PREALNPs, and ALNPs during 7-day culture. * shows p-value < 0.05

other groups. In addition, the immunomodulatory activity of RESALNPs was significantly higher than that of ALNPs and control groups.

3.5. Release assay results

Results (Figure 4) showed that resveratrol and prednisolone were released from PREALNPs and RESALNPs in a controlled manner that sustained for over 14 days. Drug release initiated by a burst release phase that continued with a slow

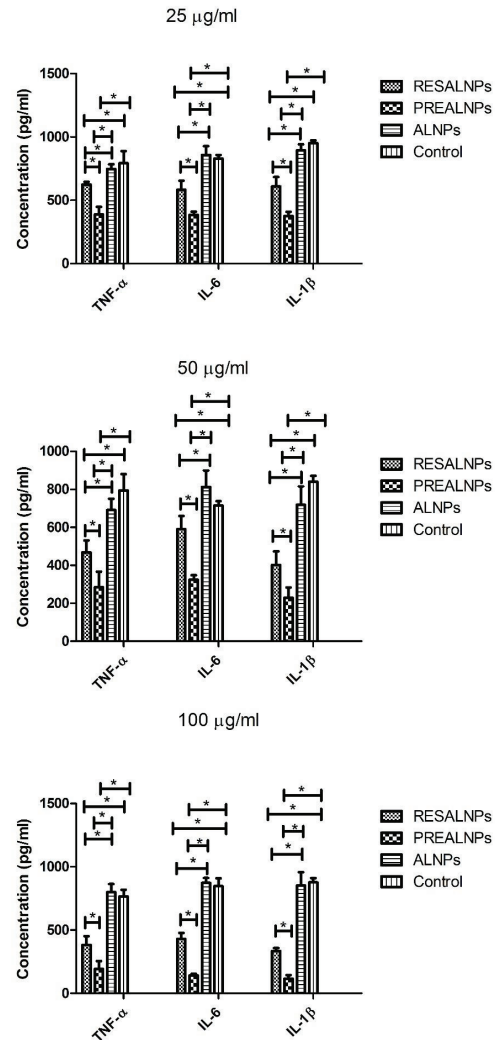


Fig. 3. Anti-inflammatory assay results with RESALNPs and PREALNPs compared with ALNPs and cells cultured with the normal media as the control group, * shows p-value < 0.05

release phase. At the end of 14th day, the cumulative release of resveratrol and prednisolone were around $68.99 \pm 3.83\%$ and $72.15 \pm 4.08\%$, respectively.

3.6. In vivo study results

3.6.1. Histological evaluations

The histopathological findings (Figure 5) from the control group revealed a range of issues in the joints, including swelling, deterioration of cartilage and bone, the formation of pannus, synovial tissue

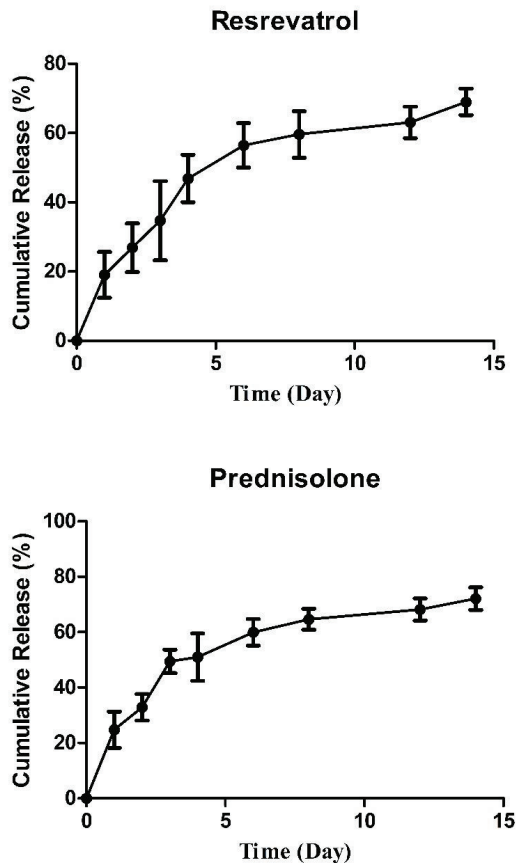


Fig. 4. Release of resveratrol and prednisolone from ALNPs during the course of 14 days in PBS

overgrowth, and inflammation, all of which were confirmed by the presence of inflammatory cell infiltration. However, in the RESALNPs and PREALNPs groups, there was a noticeable improvement in paw inflammation and joint swelling. Surprisingly, the Hybrid group exhibited a significant reduction in paw swelling when compared to the other groups. Within the RESALNPs and PREALNPs groups, there were clear signs of relief from severe paw inflammation and joint swelling. Conversely, the Hybrid group displayed a remarkable decrease in the rate of paw swelling compared to the other groups. In the control group, we also observed substantial damage to the hyaline cartilage and significant alterations in the bone structure, including severe bone degeneration. In the control group, there was evidence of synovial

hyperplasia and a significant presence of inflammatory cells, as well as the formation of pannus tissue, which ultimately led to severe damage to the cartilage. Notably, a consistent density of cartilage tissue was not detected, and the formation of pannus tissue contributed to the degradation of articular cartilage and the progression of bone lesions. In contrast, animals in the Hybrid group displayed a protective layer of smooth hyaline cartilage covering the bone surface in both treatment groups. Furthermore, we observed slight changes in the cartilage and bone layers, including an increase in cartilage thickness and a reduction in the penetration of inflammatory cells.

3.6.2. ELISA assay results

Results (Figure 6) showed that the tissue concentrations of IL-6 and TNF- α in the control group were significantly higher than other groups. The Hybrid group had significantly lower concentrations of these cytokines than other groups. TGF- β concentrations in the RESALNPs, PREALNPs, and hybrid groups were significantly higher than control groups.

4. Discussion

Nanocarriers represent a promising avenue for advancing the treatment of rheumatoid arthritis, offering a solution to the challenges associated with drug delivery in this chronic autoimmune disease [19, 20]. In the current study, resveratrol and prednisolone were administered using ALNPs to improve their bioactivity and therapeutic functions. The developed nanocarriers had a small size and narrow size distribution. The small size of ALNPs enables them to penetrate specific tissues, overcome tissue barriers, and improve drug bioavailability [20, 21]. DLS assay showed that the hydrodynamic size of all nanocarriers was slightly bigger than the size measured by the SEM images. This size reduction could be due to the lyophilization process that eliminates water layers around particles. The zeta potential of ALNPs, much like that of other nanoparticles, serves as a gauge of their surface charge. Generally, ALNPs bear a negative zeta potential, signifying an overall negative

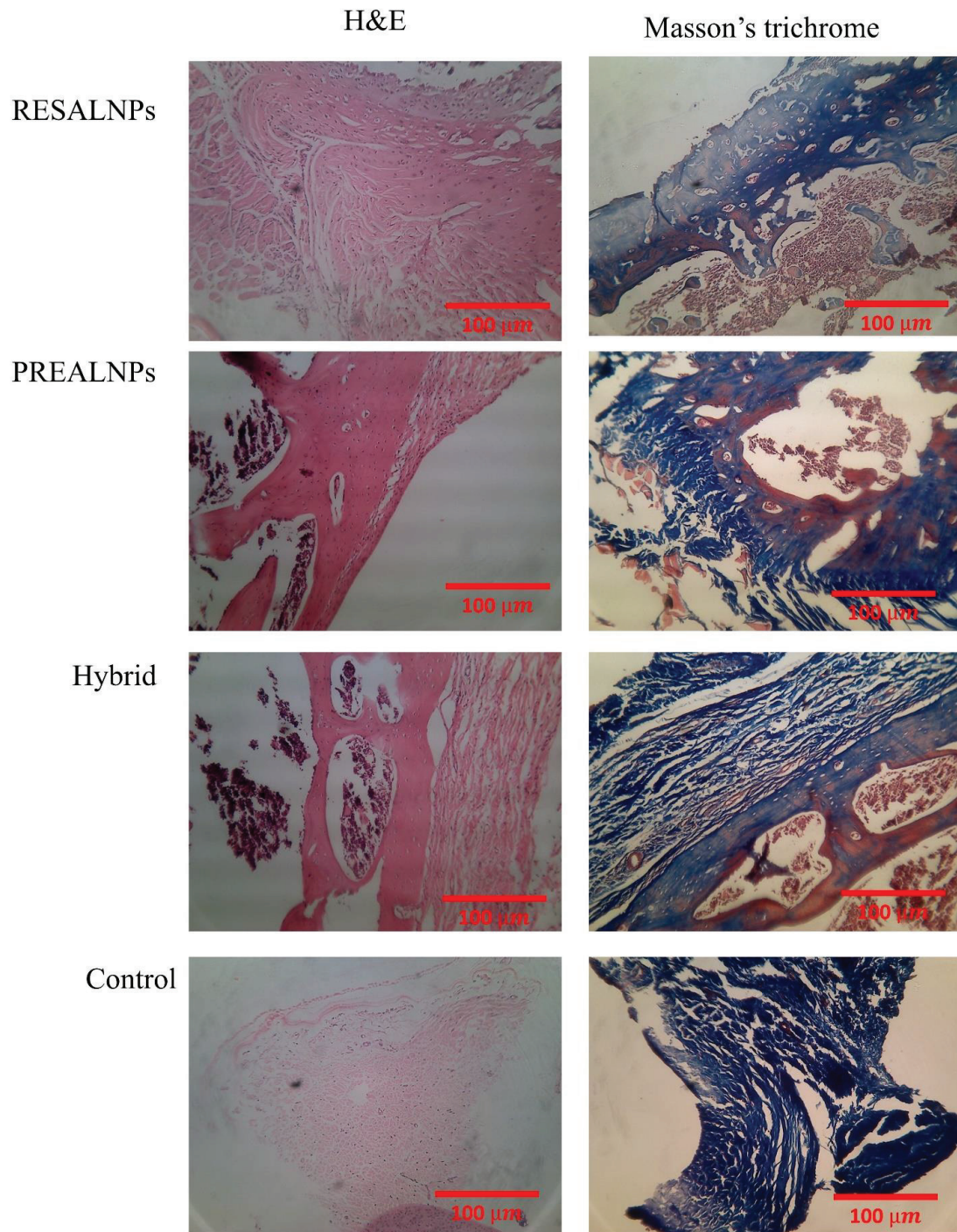


Fig. 5. Hematoxylin and Eosin (H&E) and Masson's trichrome staining images of cartilage tissue in different groups

electric charge on their surface. This stems from the ionization of specific functional groups within the albumin molecules comprising the nanoparticles.

The magnitude of this zeta potential plays a pivotal role in shaping the nanoparticles' stability and conduct in various biological and environmental

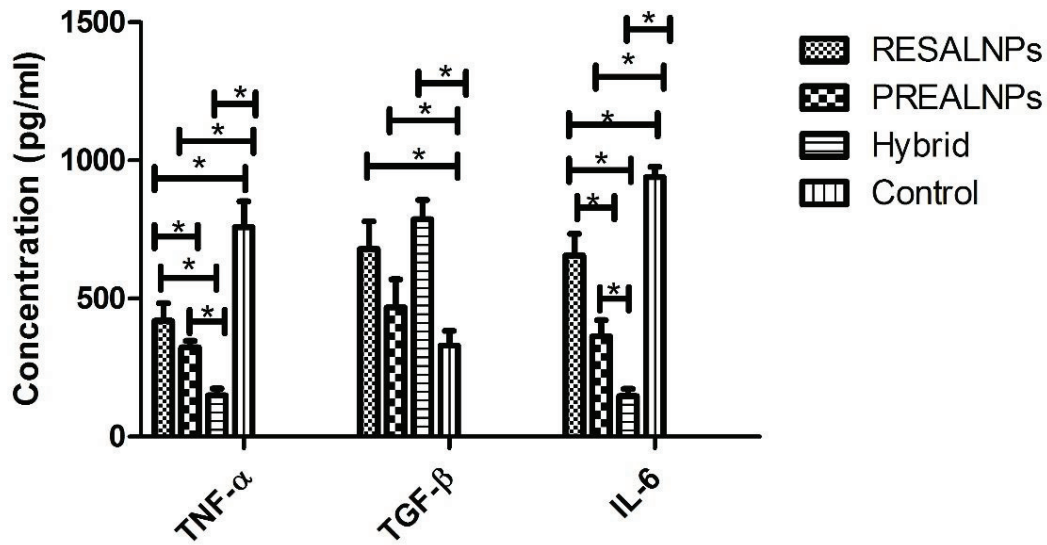


Fig. 6. Tissue concentrations of TNF- α , TGF- β , and IL-6 in cartilage tissue treated with different nanocarriers, * shows p-value < 0.05

settings. A higher absolute zeta potential often signifies increased electrostatic repulsion between nanoparticles, ultimately leading to enhanced dispersion and stability. For ALNPs, their negative zeta potential serves to deter aggregation and bolster their performance in applications such as drug delivery [22, 23]. The biocompatibility of our developed nanocarriers was validated by our MTT assay results, which is in accordance with the results of previous studies [17, 24]. The boost in cell viability in response to culture with RESALNPs can be attributed to the ability of resveratrol to enhance cell metabolic activity. Anti-inflammatory potential is crucial for any treatment strategy intended for the treatment of rheumatoid arthritis. Our results showed that both RESALNPs and PREALNPs had significantly higher immunomodulatory function compared with ALNPs and control groups. Prednisolone and resveratrol exhibit anti-inflammatory activity through different mechanisms. Prednisolone, a synthetic corticosteroid, suppresses inflammation by binding to glucocorticoid receptors, which leads to the inhibition of pro-inflammatory gene expression and the reduction of immune responses [25]. In contrast, resveratrol, a natural polyphenol, exerts its anti-inflammatory

effects by modulating various signaling pathways, including the inhibition of inflammatory mediators like NF- κ B and the activation of anti-inflammatory pathways [26]. Both compounds can mitigate inflammation, but they do so through distinct molecular mechanisms. Sustained release from ALNPs could be due to the swelling, biodegradation, or diffusion mechanisms [27]. However, in the in vivo settings, the combinations of these mechanisms may take place. Our in vivo studies showed that the healing activity of the Hybrid group was highest among experimental groups and was associated with a significant reduction in tissue concentrations of pro-inflammatory cytokines. It could be that the synergistic anti-inflammatory activity of PREALNPs and RESALNPs may have caused the observed healing functions. Resveratrol activates sirtuin 1 (SIRT1), a deacetylase enzyme that can modulate inflammation and immune responses [28]. Resveratrol's antioxidant properties further protect against oxidative stress, which plays a role in rheumatoid arthritis pathogenesis. On the other hand, prednisolone, being a corticosteroid, operates by attaching to glucocorticoid receptors found within cells. This attachment hinders the activation of pro-inflammatory

transcription factors, such as NF- κ B and AP-1, which results in the inhibition of pro-inflammatory gene expression [29, 30]. As a consequence, it reduces the generation of inflammatory cytokines and immune responses. When used together, prednisolone and resveratrol may offer a more comprehensive approach to rheumatoid arthritis management. Prednisolone provides potent and rapid immune suppression, while resveratrol enhances anti-inflammatory effects and counteracts oxidative stress.

5. Conclusion

In summary, our current research has successfully harnessed the therapeutic potential of resveratrol and prednisolone by encapsulating them within ALNPs, marking a significant step forward in the quest for an effective treatment for rheumatoid arthritis. The resulting nanocarriers proved to be both non-toxic and adept at modulating inflammatory responses, laying a solid foundation for their application in targeted drug delivery. The compelling outcomes of our *in vivo* study underscore the tangible benefits of our approach, as animals treated with resveratrol and prednisolone-loaded ALNPs exhibited markedly improved alleviation of hind paw joint issues. This promising observation not only speaks to the efficacy of our developed nanocarriers but also suggests a potential breakthrough in enhancing the therapeutic outcomes for rheumatoid arthritis sufferers. Complementing the *in vivo* findings, the results from the ELISA assay provide valuable insights into the underlying mechanisms of our treatment. The implication that our developed therapy may have contributed to the reduction of inflammatory reactions at the injury site further solidifies the potential clinical relevance of our approach. Moving forward, our research will refine ALNPs encapsulating resveratrol and prednisolone for enhanced rheumatoid arthritis treatment. We will optimize the nanocarrier design, explore new drug combinations, and conduct thorough preclinical validation. These efforts aim to ensure safety, efficacy, and long-term benefits in animal models. Following successful preclinical studies, we will progress to clinical trials, starting

with safety assessments in Phase I and efficacy evaluations in Phase II. Our ultimate goal is to deliver personalized medicine, offering tailored treatments that significantly improve the lives of rheumatoid arthritis patients.

Conflicts of interests

None

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